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EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

VOLUME 46, NUMBER 8 AUGUST, 1958

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PHYSIOLOGY

INTEROCEPTIVE REGULATION OF BLOOD COAGULATION

COMMUNICATION III

REFLEX INFLUENCE OF ANTIGENIC STIMULI ON BLOOD COAGULATION

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(Received November 13, 1957. Presented by Active Member AMN SSSR V. N. Chernigovskii)

We have shown in previous communications reflex changes in blood coagulation can be obtained by action of chemoreceptors and baroreceptors in the carotid sinus and the arch of the aorta.

Acceleration of blood coagulation has been obtained by A. L. Komendantova [7] on presenting a resolving dose of antigen simultaneously to intestinal and spleen receptors. Slowing of blood coagulation was observed by A. N. Gordienko [3] and T. A. Nazarova [8] when peptone and resolving dose of antigen were allowed to act on the carotid sinus zone. However, experiments with foreign serum acting on the carotid sinus zone [8] were carried out without analgesia, and fear and pain are known to be accompanied by considerable changes in blood coagulation; moreover, the stimulus was introduced into the carotid sinus sac by puncturing the wall of the carotid sinus – a procedure leading to powerful stimulation of baroreceptors. E. N. Budigaev [2] found that anesthesia of the carotid sinuses had no effect on slowing of blood coagulation in peptone shock.

Since altered, allergic reactivity of the organism is thought to be implicated [6, 12 and others] in the pathogenesis of diminished blood coagulability and of some types of hemorrhagic diathesis, especially those associated with excess of antithrombin, we decided to investigate anew the reflex influence of antigens on blood coagulation in the organism with unchanged and with altered immunobiological reactivity, and also to trace the dynamics of changes in blood coagulation, which had not been done by earlier investigators.

EXPERIMENTAL METHOD

A total of 54 experiments was performed, of these 10 on normal and 44 on sensitized dogs. Dogs sensitized with horse serum given subcutaneously in amounts 0.2 ml/kg body weight on 2 successive days were subjected to experiments 15-25 days after administration of antigen. The methods of analgesia, perfusion of the carotid sinus and collection of blood specimens have been described previously [4]. Coagulation time was determined by the Fonio method and in Bazaron's apparatus. The carotid sinus receptors were acted upon by whole horse serum (1 ml) and solutions of nicotine and acetylcholine in concentrations from 10^{-4} to 10^{-7} in volumes of from 0.25 to 1 ml.

In experiments with intravenous administration of resolving dose of antigen to sensitized animals simultaneous recordings were made of blood pressure, respiration and blood coagulation rate. All cases presented a picture of anaphylactic shock. The absence of desensitization indicated complete isolation of the carotid sinus from the general circulation.

EXPERIMENTAL RESULTS

In the first series of experiments blood coagulation time was studied during introduction of horse serum into the perfusion fluid of the carotid sinus in unsensitized dogs. No reflex changes in blood coagulation were obtained in most of the experiments in this series, but toward the end of the experiments coagulation time usually diminished (Fig. 1). This is evidently connected with the effect of anesthesia, liberation of thromboplastin from damaged tissues and some degree of anemia of the carotid sinus receptors.

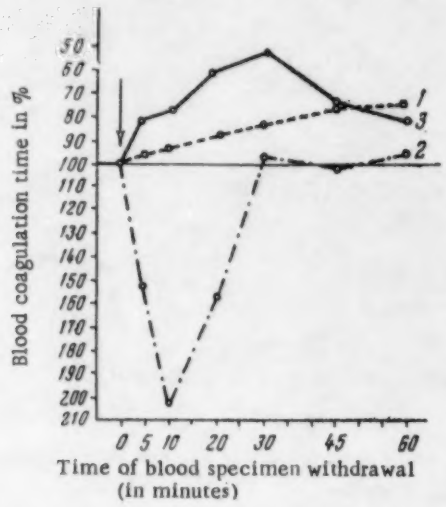

Fig. 1. Rate of blood coagulation during action of horse serum on the carotid sinus of unsensitized dogs (1), sensitized dogs (2), during stimulation of carotid sinus chemoreceptors in sensitized dogs with nicotine and acetylcholine solutions (3). ↓ beginning of stimulation.

In one of 7 experiments introduction of horse serum led to slight increase in the amplitude of respiratory movements and some decrease in blood coagulation time, but the latter was much less marked in degree and duration than the effect produced by stimulation of chemoreceptors by nicotine or acetylcholine. Intactness of the neural connections of the carotid sinus was checked after all the experiments by reflex change in respiration in response to administration of acetylcholine solution.

In the second series of experiments a study was made of the change in blood coagulation time after introduction of a resolving dose of antigen into the fluid perfusing the carotid sinus of sensitized dogs. In most of these experiments coagulation time showed an initial increase. It doubled, on the average, in the course of 10 minutes. After this it began to diminish, approaching the initial value. The period of slowed coagulation fluctuated in individual experiments within the range of 10 to 60 minutes.

In 6 out of 10 experiments the initial delay of blood coagulation was followed by its acceleration, the coagulation time becoming even shorter than the initial, a phenomenon not usually observed in general anaphylactic shock. In one case transient slowing of blood coagulation was followed by definite acceleration of the latter, as in experiments with stimulation of carotid body receptors by chemical agents.

On the whole the reflex changes in blood coagulation observed were considerably smaller than slowing of blood coagulation seen in general anaphylactic shock in the same animals.

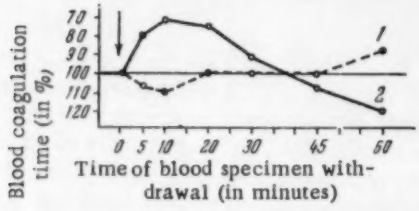

Fig. 2. Rate of blood coagulation in vagotomized dogs under the influence of a resolving dose of horse serum on the denervated carotid sinus (1) and on carotid sinus with intact sinus nerve (2).

Assessment of mean results reveals only reflex slowing of blood coagulation (Fig. 1), while the acceleration phase is blurred by individual variations.

Since vascular isolation of the carotid sinus does not give complete guarantee of absence of antigen resorption into the general circulation, control experiments were carried out with determination of the antigen in the blood after its introduction into the carotid sinus perfusate. When precipitation serum with a titer of 1:30,000 was used it was found that with the method employed not even 0.005 ml horse serum penetrated into the general circulation. However, in the absence of greater assurance, we injected sensitized dogs in 4 experiments with such amounts of horse serum (from 0.005 to 0.4 ml, intravenously) as were known to exceed the amount which could penetrate from the perfusing fluid. It was found that small doses of specific serum produced no substantial changes in blood coagulation in sensitized dogs. Deviations of coagulation time in these cases were even smaller than in control experiments in which the carotid sinus was perfused without being stimulated.

In order to study the specificity of the slowing of blood coagulation obtained in response to stimulation of carotid sinus zone receptors by foreign serum a third series of experiments was staged in which 10 sensitized dogs were subjected to perfusion of the carotid sinus with fluid containing stimulating substance of nonantigenic nature: acetylcholine and nicotine. The mean curve (Fig. 1) illustrating these 10 experiments shows that when the chemoreceptors are stimulated by acetylcholine or nicotine the coagulation time is nearly halved. As in nonsensitized dogs, the blood coagulation rate reaches the greatest value on the 20th-30th minute after beginning of stimulation; increased coagulability persists for about 1 hour.

On the basis of references [13, 5, 9 and others] to the significance of parasympathetic innervation in slowing blood coagulation a fourth and fifth series of experiments were carried out on sensitized dogs with transsected cervical vagi. The time which elapsed from the moment of vagal transection to the beginning of blood coagulation time determination was about 2 hours.

The fourth series of experiments (10) was a control one. In this series blood coagulation time was studied in vagotomized dogs after introduction of a resolving dose of antigen into the fluid perfusing the denervated carotid sinus and during perfusion of the carotid sinus without stimulating agents. No consistent changes in blood coagulation time were discovered in these experiments (Fig. 2).

In the fifth series the action of resolving dose of antigen on intact carotid sinus receptors of vagotomized dogs produced no appreciable change in blood coagulation time in 2 experiments out of 10 and definite acceleration in the remaining 8 which was, however, considerably less in degree and duration than that observed on stimulation of carotid sinus receptors by chemical stimuli. The curve representing the mean results of 8 experiments is shown in Fig. 2.

Our data and some of the literature data [1, 11,etc.] suggest that reflex slowing of blood coagulation in sensitized dogs observed when a resolving dose of antigen is allowed to act on carotid sinus receptors is mediated by excitation of the vagal center through stimulation of depressor baroreceptors of the carotid sinus. Increase of tone in smooth muscle of sensitized animals under the influence of specific serum is a well-established fact [14, 10,etc.]. Contraction of the carotid sinus walls is as real a stimulus as a rise in hydrostatic pressure [11].

This explains the reason why our data coincide with E. Perlik's [12] observations that increase in pressure in the carotid sinus leads to a definite increase in the concentration of heparin–antithrombin with simultaneous decrease in the concentration of prothrombin, factor V and factor VII.

SUMMARY

In the majority of cases injection of horse serum into the perfusate of the carotid sinus of normal dogs causes no reflex change of coagulation time. However, if the same stimulus is administered to previously sensitized dogs a reflex increase of coagulation time takes place. This prolongation is most pronounced in 10 minutes. In a number of cases, this delay in blood coagulation is replaced by its acceleration. Preliminary vagotomy removes reflex delay of blood coagulation.

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•In Russian.

••Original Russian pagination. See C. B. Translation.

CHARACTERISTICS OF COMPLEX REFLEX REGULATION OF BLOOD SUGAR LEVELS IN MAN

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(Received June 14, 1956. Presented by Active Member AMN SSSR V. N. Chernigovskii)

It was shown in a previous communication [1] that sham feeding of animals with gastrostomy and esophagogastrostomy led to a prolonged rise of blood sugar and gas exchange. This suggested a complex reflex mechanism of carbohydrate metabolism regulation. However, this fact, established on animals, required similar observations on man for its more precise definition.

The present communication deals with the study of the effect of sham feeding and of other means of introducing food on regulation of blood sugar levels in man.

Investigations were carried out at the otolaryngologic clinic of the Leningrad Medical Stomatologic Institute, directed by Professor N. A. Karpov* on patients who had been subjected to therapeutically indicated extirpation of the pharynx and sometimes of the larynx. Postoperatively the patients had an esophagostomy which disconnected the oral cavity from the stomach. These patients are fed for some time postoperatively through a stomach tube introduced into the lower aperture of the esophagus or through a fine tube introduced into the stomach by way of the lower nasal passage. This makes possible separate investigation in these patients of the effect of sham intake of carbohydrate on the blood sugar level and the effect of the same substances on the blood sugar on being introduced directly into the patient's stomach.

METHOD

Investigations were performed on 16 patients. All the patients had completely recovered from the operation by the time of the experiments, were in satisfactory condition, had an adequate appetite and moved freely about the clinic.

Observations were made in the morning, in the fasting state. The sterile dressing was removed from the esophagotomy aperture and the patient was given 500 ml warm tea sweetened with 50 g glucose. While the patient was drinking, a vessel was placed so as to collect all the liquid drunk as it escaped from the esophageal aperture. The tube and cotton-wool tampon present in the lower part of the esophagus disconnected the latter from the upper part of the esophagus and precluded any possibility of the ingested liquid gaining acess to the stomach. The total period of sham drinking usually lasted 5-6 minutes. Fasting blood sugar level was determined prior to the sham ingestion of glucose and the blood sugar examinations were repeated 5-10-20-30-45-60-90-120-180 minutes after the sham intake of glucose. Blood sugar estimations were made by the Hagedorn-Jensen method.

All the patients participating in these observations drank the sweet tea with pleasure and at the end of this sham intake spoke of the pleasant gustatory sensation experienced. The patients mentioned that the postoperative absence of feeding by mouth was trying and were very happy to experience again the partially forgotten pleasure associated with normal eating.

* The author takes this opportunity for expressing his profound gratitude to Professor N. A. Karpov and members of the clinic G. M. Smerdov and I. I. Liubavina for their help in this work.

RESULTS

The results of the observations are presented as mean data in Fig. 1, a, and show that sham ingestion by the patients of 10% solution of glucose is followed within 5 minutes by a rise in blood sugar level. The blood sugar content continues to rise, reaching a maximum usually 20 minutes after the sham feeding. This raised level is maintained for 10-30 minutes, after which a gradual decline begins. The blood sugar content remains above the initial level for the next 2 hours, although in isolated cases there are deviations from the mean value.

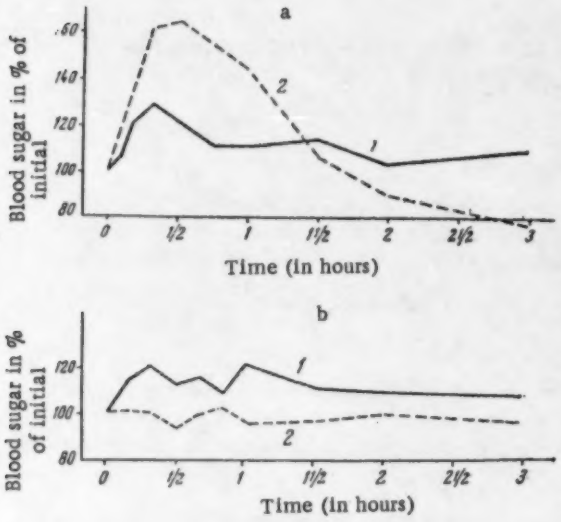

Fig. 1. Effect of sham feeding (—) and introduction through the esophagostomy (— — —) of solutions on blood sugar content in patients. a) 500 ml 10% glucose solution; b) 500 ml 0.01% solution of saccharin.

Sham glucose ingestion thus gives rise to hyperglycemia, despite the fact that the patient can see that all the ingested fluid is completely removed; this hyperglycemia persists throughout the 3 hours of observation. This suggests that the ingestion of glucose exerts a complex reflex action on blood sugar content in man. The observed rise in blood sugar under these circumstances is, of course, connected with specific stimulation of oral receptors which leads to increase in blood sugar content [2].

When the procedure is repeated (such investigations were carried out on 3 patients) the hyperglycemic effect is diminished (Fig. 2, a), i.e., extinction of the natural conditioned food reflex takes place.

The effect of glucose solution, introduced directly into the stomach, on blood sugar content was studied in 11 patients under observation. The patients were unaware of the nature of the fluid being introduced into the stomach and were under the impression that it was water. The results of these investigations, presented in Fig. 1, a, show that introduction of glucose solution through the esophagostomy into the stomach also causes a rise of blood sugar which begins in most of the patients in question 10 minutes after introduction and reaches a maximum within 30 minutes. The blood sugar level returns to the initial within 1 hour and drops below that by the end of the period of observation. The period of hyperglycemia following introduction of glucose through the esophagostomy into the stomach thus lasts $1\frac{1}{2}$ hours, dropping to an average of 21.4% below the initial blood sugar level within the next $1\frac{1}{2}$ hours. The blood sugar content curve is diphasic following introduction of glucose solution directly into the stomach: the first phase is hyperglycemic and is associated with absorption and passage of glucose into the blood, the second is hypoglycemic and is, evidently, associated with increased absorption of sugar by tissues and its combustion, or perhaps with increased secretion of insulin. Thus in summary, the blood sugar curve is monophasic in sham feeding with glucose and diphasic when glucose solution is introduced through the esophagostomy.

The work of I. S. Kanfor and R. P. Olnianskaia [2] on the role of oral receptors in the regulation of carbohydrate metabolism demonstrated the effect of ingestion of saccharin on blood sugar content and on gas exchange. These observations were made on healthy subjects. It was important to re-examine this question on patients, making a study of the effect of saccharin intake both in sham feeding and in direct introduction through the esophagostomy.

These experiments were performed on 5 patients. Saccharin was given in 1:10,000 solution in amounts of 500 ml. Sham ingestion was accompanied by a sensation of sweet taste, whereas when saccharin was introduced directly into the stomach through the tube, the patient was unaware of the composition of the fluid.

The results of these observations show that sham drinking of saccharin solution produces increased blood sugar content throughout the whole 3-hour period of observation. Introduction of saccharin solution through

the esophagostomy directly into the stomach produced no rise in blood sugar content. Comparison of blood sugar curves in sham ingestion of saccharin solution and in its introduction through the esophagostomy is clearly illustrated in Fig. 2, b.

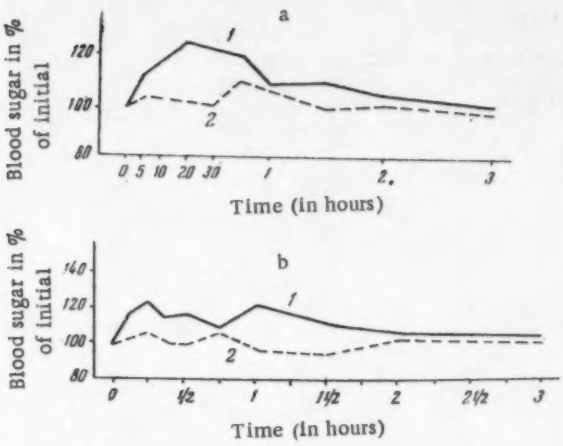

Fig. 2. Effect of first (—) and repeat (– – –) sham ingestion of solutions on blood sugar content in patients. a) 500 ml 10% glucose solution; b) 500 ml 0.01 % saccharin solution (mean data of all observations).

These data indicate that the hyperglycemic action of saccharin is only manifested when it enters the organism by way of the oral cavity. It follows from this that this action of saccharin is associated with stimulation of taste receptors in the mouth; in the absence of this condition, when saccharin is introduced directly into the stomach, its ability to elicit a rise in blood sugar content is abolished.

The material presented provides a basis for assuming that the rise in blood sugar seen after drinking saccharin solution is conditioned reflex in nature and is connected with excitation of peripheral receptors of taste nerves in the mouth.

Repeat sham drinking of solutions of saccharin and of glucose produces a smaller hyperglycemic effect (Fig. 1, a and b). It follows from this that conditioned reflex increase in blood sugar content which develops after sham ingestion of glucose and saccharin solutions is unstable and quickly extinguished. Comparison of the results of sham ingestion of saccharin and of glucose solution shows no essential difference in the dynamics of blood sugar content.

The opinion expressed in the literature that a hyperglycemic effect can only be obtained following administration of saccharin if it acts on gastric receptors [3, 4] is, in our view, insufficiently well founded. The hyperglycemic effect of saccharin ingestion, as shown by our observations, develops predominantly when the oral taste receptors are stimulated in the act of ingestion.

In summary, the data presented permit the conclusion that regulation of blood sugar content is mediated by a complex reflex route. The most important role in this process is the act of eating the carbohydrates itself, which achieves excitation of oral receptors and this in turn elicits, through the afferent system, complex reflex stimulation of carbohydrate metabolism. This complex reflex rise in blood sugar level exerts an influence on the dynamics of carbohydrate metabolism generally.

The presence of food in the mouth thus plays an extremely important part both in the process of digestion and in metabolism. Despite the short time during which food is present in the mouth, important biologic processes take place during the act of eating; these processes consist of reflex excitation of the whole glandular digestive apparatus, stimulation of carbohydrate metabolism and enhancement of oxidative processes in tissues, which in its turn leads to enhancement of the whole energy turnover.

SUMMARY

Sham feeding of glucose solution and saccharin to patients after esophagotomy causes prolonged (3 hours) rise of blood sugar of almost equal degree. Administration of glucose solution through the esophagostomy directly into the stomach causes an increase in blood sugar for 1½ hours with subsequent fall below (by 21.4%) the initial level. Administration of saccharin solution into the stomach and through the esophagostomy causes no rise in blood sugar level whatsoever. The results which were obtained emphasize the significance of the act of eating as the leading link in the control of blood sugar in man. Thus, it was shown that the control of blood sugar is effected in a complex reflex way, including both unconditioned and conditioned natural food reflexes.

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*In Russian.

DYNAMOCARDIOGRAPHIC EXAMINATION OF HEALTHY CHILDREN AGED 8 TO 15 YEARS

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(Received February 13, 1957. Presented by Active Member AMN SSSR A. N. Bakulev)

The new method of investigation of cardiac activity – dynamocardiography (originally named cardiohemodynamography) – was described by E. B. Babskii et al., in 1952 [1]. A number of reports has now appeared on dynamocardiographic studies of patients with various cardiac diseases. However, no special studies of dynamocardiograms in healthy subjects have so far been published.

We needed to know the normal dynamocardiographic characteristics of healthy subjects within the age group of 8 to 15 years for our studies on the dynamocardiographic features exhibited by children suffering from transposition of the great vessels. With this aim in view, we examined 58 healthy schoolchildren within the above age group attending one of the Moscow schools. Conditions of dynamocardiogram recording were the same as those described previously [2, 3].

EXPERIMENTAL METHOD AND RESULTS

The state of health of the children examined was determined from data on their past history, general physical examination, percussion and auscultation of the chest and reports by the school medical officer on each child. Of the 58 children investigated 29 were boys and 29 girls.

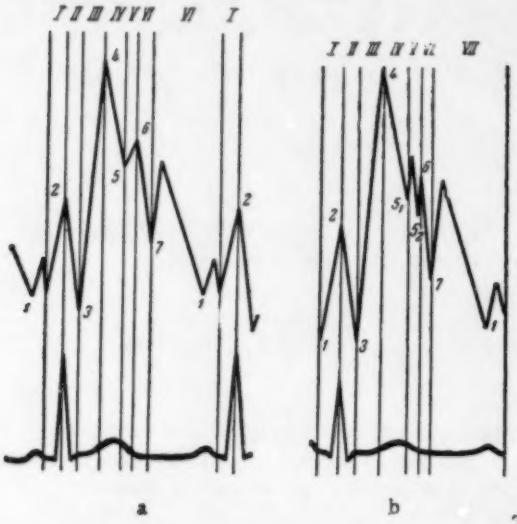
The normal dynamocardiogram of healthy adults, recorded at a film speed of 10 mm/sec, has the appearance of a serrated line (Fig. 1) on which intervals described by the authors [4] and corresponding to the different phases of the cardiac cycle can easily be distinguished.

The first phase of ventricular systole – phase of isometric contraction – is reflected in the dynamocardiogram by the portion of the curve in the interval II. In children this has the form of a straight line sloping downward.

The subsequent phases of ventricular systole – phases of rapid and slow expulsion – are represented on the dynamocardiogram by intervals III and IV. In interval III (rapid expulsion) the curve rises sharply and then, in interval IV (slow expulsion), slopes down to some extent.

In 25 of our observations the curve was notched at the very beginning of interval III with a small notch of low amplitude. The position of this notch was very inconstant. It was most frequently found between the end of interval II and beginning of interval III. In some cases the same subject showed this notch in different positions during different cycles. In such instances it was always referred to the phase of expulsion. Since the presence of this small notch at the beginning of interval III in the dynamocardiogram is observed in healthy children (and in healthy adults) we suppose that this feature is one of the normal dynamocardiographic variants. The origin of this small notch in interval III is not as yet quite clear.

Interval IV is little variable. Interval V corresponds to the protodiastolic period. It is represented by a small portion in the form of a straight line sloping upwards. In 16 observations two protodiastolic periods were noted, following each other in very quick succession. We tend to regard this as asynchronous closure of the semilunar valves of the aorta and pulmonary artery. For convenience of analysis of the dynamocardiogram we distinguish points 5_1 and 5_2 in connection with the presence of two protodiastolic periods, and intervals V corresponding to them (Fig. 1, a and b).



a b

Fig. 1. Schematic drawing of a normal dynamocardiogram. a) Without splitting of the protodiastole, b) with splitting of the protodiastole ($5_1 5_2$). Arabic numerals indicate dynamocardiogram notches. Roman numerals denote the dynamocardiogram intervals. Interval I corresponds to auricular systole, interval II corresponds to the phase of isometric contraction of the ventricles. Interval III corresponds to the period of rapid expulsion of blood from the ventricles and interval IV to the period of slow expulsion, interval V corresponds to the protodiastolic period, interval VI to period of relaxation, interval VII to period of ventricular filling with blood. Intervals II, III, IV corresponding to ventricular systole, intervals I, V, VI, VII correspond to ventricular diastole.

The next phase in diastole – phase of relaxation – is represented in the dynamocardiogram by interval VI. During this phase the portion of the dynamocardiogram appears as a descending straight line; no other changes have been observed in this part of the dynamocardiogram. Interval VII (phase of filling) has the appearance of a complex broken line, following first an ascending course, then descending, which is evidently connected with rapid and slow filling of the ventricles with blood. Interval I corresponds to auricular systole. This part of the dynamocardiogram is an ascending straight line. In most cases the dynamocardiogram intervals thus have the appearance of straight line segments of a complex broken curve. Four variants of the normal dynamocardiogram for children aged 8 to 15 years can be distinguished on the basis of the facts presented above (Fig. 2).

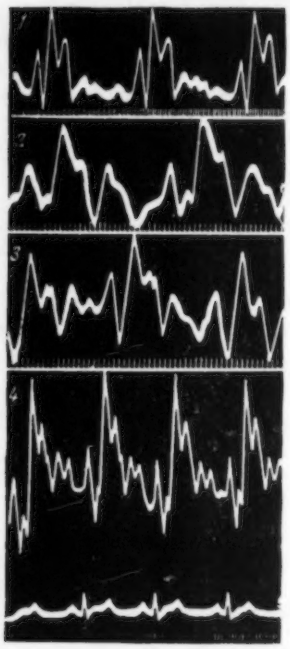


Fig. 2. Variants (1-4) of the normal dynamocardiogram in children aged 8 to 15 years. The electrocardiogram is shown at the bottom of variant 4 as a marker. Time marker 0.05 second.

No changes in any intervals were observed in 25 cases, i.e., 43.1%. Notching in interval III was seen in 16 cases, 26.7%. Splitting of the curve in interval V was noted in 6 cases, i.e., 10.4%. Changes in intervals III and V were seen in 11 cases, i.e., 18.9%.

The amplitudes of the curve during the phase of expulsion of blood from the ventricles and during the phase of contraction were compared for the purposes of amplitude analysis of the dynamocardiogram. These amplitude ratios were expressed as an amplitude coefficient, taking the amplitude during the phase of contraction tion as unity. The amplitude coefficient of the normal dynamocardiogram in children aged 8 to 15 years is 1.8. In adults it equals 2.3 (V. L. Karpman). The 4 variants of the normal dynamocardiogram have approximately the same amplitude coefficient.

Comparison of the dynamocardiogram in healthy children aged 8 to 15 years and in adults with respect to pattern reveals no

significant difference. There is only some decrease of amplitude during the phase of blood expulsion in children as compared with adults, which is evidently associated with the smaller volume of blood expelled.

TABLE 1

Mean Duration of Individual Phases of the Cardiac Cycle in Children Aged 8 to 15 Years According to Dynamocardiographic Data

DCG intervals	Phases of the cardiac cycle	Duration in seconds		
		arithmetic mean (M)	mean error of arithmetic mean (± M)	mean square deviation (± σ)
II	Phase of contraction	0,071	0,002	0,013
III	Phase of rapid expulsion	0,113	0,002	0,013
III+IV	Expulsion as a whole	0,210	0,002	0,017
II+III+IV	Ventricular systole	0,281	0,003	0,025
V	Protodiastolic period	0,046	0,001	0,015
VI	Phase of relaxation	0,072	0,002	0,014
VII	Phase of filling	0,090	0,010	0,093
I	Auricular systole	0,089	0,001	0,010
V+VI+VII+I	Ventricular diastole	0,523	0,010	0,100
I—VII	Cardiac cycle	0,800	0,010	0,109

TABLE 2

Limits of Variation of Duration of the Individual Phases of the Cardiac Cycle According to Literature Data and Dynamocardiographic Data

Phases of cardiac cycle	Limits of variation of the duration of individual phases of the cardiac cycle in seconds	
	according to data of various authors	according to present author's data
Ventricular systole	0,19—0,39	0,24—0,34
Phase of contraction	0,14—0,10	0,05—0,10
Phase of rapid expulsion	—	0,08—0,14
Expulsion of blood from ventricles as a whole	0,15—0,36	0,17—0,26
Ventricular diastole	—	0,35—0,71
Protodiastolic period	0,07—0,10	0,02—0,07
Phase of relaxation	0,07—0,10	0,05—0,11
Phase of filling	—	0,011—0,52
Auricular systole	0,09—0,14	0,07—0,10

The mean error of the arithmetic mean and the mean square deviation were determined. The duration of all the phases of the cardiac cycle in children aged 8 to 15 years as shown by the dynamocardiogram data is presented in Table 1.

It should be noted in connection with the data shown in Table 1 that in the case of two protodiastolic periods being present the duration of the expulsion phase was considered to be more correctly estimated up to point 5_2. If the duration were calculated to point 5_1 it would give the time necessary for expulsion of blood only from that ventricle which achieved the task first. If, however, the duration of the expulsion phase is calculated to point 5_2, the time needed for expulsion of blood from both ventricles is determined.

Moreover, when the dynamocardiogram is recorded simultaneously with the electrocardiogram the point 5_2 corresponds in time to the end of electric systole, the T-wave. If the dynamocardiographic data on the expulsion phase calculated to point 5_1 and point 5_2 are compared with literature data a more satisfactory agreement is found to exist in the latter case.

Consequently, everywhere two protodiastolic periods were present on the dynamocardiogram the duration of the expulsion phase was calculated to point 5_2.

TABLE 3

Mean Duration of the Individual Phases of Cardiac Contraction Depending on the Total Duration of the Cardiac Cycle in Children Aged 8 to 15 Years

Phases of the cardiac cycle	Duration of individual phases of cardiac contraction when the total duration of the cycle is				
	0,60—0,69	0,70—0,79	0,80—0,89	0,90—0,99	1,00—1,09
Ventricular systole	0,26	0,27	0,28	0,31	0,31
Phase of contraction	0,06	0,07	0,07	0,08	0,08
Phase of rapid expulsion	0,11	0,11	0,11	0,11	0,12
Expulsion of blood from ventricles as a whole	0,20	0,20	0,21	0,23	0,23
Ventricular diastole	0,36	0,47	0,53	0,62	0,70
Protodiastolic period	0,03	0,04	0,05	0,05	0,05
Phase of relaxation	0,07	0,07	0,07	0,07	0,08
Phase of filling	0,18	0,27	0,32	0,41	0,47
Auricular systole	0,08	0,09	0,09	0,09	0,10
Rhythm of cardiac contractions	95	82	76	65	61

TABLE 4

Duration of Intervals of Individual Phases of the Cardiac Cycle in Children of Different Age Groups

Phases of cardiac cycle	Age groups		
	from 8 to 11 years	from 12 to 15 years	from 20 to 60 years (V. L. Karpman)
Ventricular systole	0,275	0,281	0,301
Phase of contraction	0,070	0,073	0,074
Phase of rapid expulsion	0,109	0,112	0,112
Expulsion of blood from the ventricles as a whole	0,205	0,208	0,227
Ventricular diastole	0,495	0,545	0,579
Protodiastolic period	0,048	0,045	0,032
Phase of relaxation	0,071	0,075	0,093
Phase of filling	0,288	0,335	0,376
Auricular systole	0,089	0,090	0,078
Duration of cycle	0,771	0,826	0,880

We have found a limited number of reports in Soviet and foreign literature on analysis of the individual phases of cardiac contraction in healthy subjects. These were usually arrived at from various and most often complex methods of investigation. We could find no data on the duration of the individual phases of the car-

diac cycle in healthy children within the age-group of 8 to 15 years. We could therefore only compare the values obtained by us for the duration of the cardiac cycle phases in adolescents with data obtained on adults. Since different authors give highly divergent mean values of duration of the individual phases of the cardiac cycle we took for comparison the limits of fluctuation of these values and presented them in the form of Table 2.

The certain discrepancy between the duration of the individual phases of the cardiac cycle as determined by dynamocardiography and the data of other authors can be explained by the differences in the rhythm of cardiac activity, since it is known that the duration of the individual phases of cardiac contraction depends on the total duration of the cardiac cycle. This dependence is presented in Table 3, based on dynamocardiographic data.

The results of our investigations, summarized in Table 4, suggest some relation of the duration of individual phases of the cardiac cycle to the age of the children.

SUMMARY

Dynamocardiography (cardiohemodynamography) gives an opportunity to study the hemodynamics and to judge the contractility of myocardium. Temporal analysis of all phases of cardiac activity may be carried out by the method of dynamocardiography. Fifty-eight healthy boys and girls aged 8 to 15 years were examined. The specific features of their dynamocardiograms were studied with relation to age.

The normal dynamocardiogram of healthy children has the form of a complex broken line with a characteristic pattern. There are 4 variants of the normal dynamocardiogram in children.

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OPTIMUM AND PESSIMUM FREQUENCY OF STIMULATION FOR THE TETANIC AND TONIC REFLEX APPARATUS

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E. K. Zhukov and O. V. Tarushkin [2] have suggested that the nerve centers innervating tonic skeletal muscles show slight mobility (lability). If this is the case, then tonic motor apparatus should tend to transition to a state of pessimal inhibition more than centers which innervate phasically active systems. The present work presents investigations concerned with this problem.

EXPERIMENTAL METHOD

Experiments were performed on 50 spinal frogs, one hour after the operation. The sciatic nerve was isolated on one side, with transection of the tibial and peroneal branches. The central ends of these nerves were subjected to simultaneous tetanizing stimulation, using condenser discharges interrupted by a neon breaker. The frequency of the discharges could be varied from 10 to 500 per second, without affecting the strength of stimulation. On the side contralateral to the nerve the tetanic sartorius muscle and the mixed ilio-tibial and gastrocnemius muscles were dissected and the tonic bundle of the iliofibular muscle was isolated. Muscular contractions were recorded on a kymograph. The experimental results were similar in the vast majority of experiments.

EXPERIMENTAL RESULTS

In a freshly prepared sartorius muscle the reflex responses to optimal strength of stimulation are small in amplitude and duration when the frequency of stimulation is 50-100 per second. However, even under these optimal conditions of stimulation tetanus is not usually maintained for longer than 10-15 seconds; despite the continuing stimulation of the afferent nerve the muscle undergoes relaxation fairly rapidly (Fig. 1, a). In these cases "post-inhibition excitation" is observed as a rule. At higher frequencies of stimulation reflex tetanus becomes weaker and of less duration. At stimulation frequency of 150-200 per second reflex responses are often completely absent.

The reflex responses of the tonic bundle of the iliofibular muscle ate highest in amplitude and longest in duration at a stimulation rate of 25-50 per second. Under these optimal conditions tonic contraction may be maintained without slackening for several minutes (Fig. 1, b). On termination of stimulation the muscle does not relax immediately but does so gradually; no contraction of the "post-inhibition excitation" is observed. The amplitude of tonic contraction diminishes at stimulation frequencies of 60-70 per second and higher; the contraction loses its persistence and becomes a small slow wave which drops to zero during a few seconds of stimulation.

It is noteworthy that complete cessation of reflex response of the tonic bundle occurs, as a rule, at higher frequencies of stimulation (300-350 per second) than cessation of response of the tetanic sartorius muscle (150-200 per second). Figure 2 shows reflex contractions of the whole iliofibular muscle. In response to stimulation

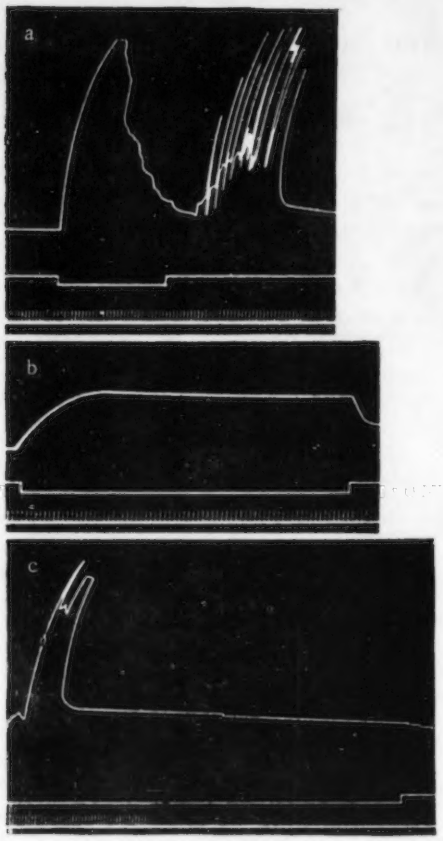

Fig. 1. Reflex contractions of frog skeletal muscles in response to optimal strength and frequency of stimulation of the sciatic nerve. a) Sartorius muscle, b) tonic bundle of iliofibular muscle, c) whole iliofibular muscle. Records from above down: myogram, time marker (1 second).

frequency of 50 per second there is first strong tetanic contraction, which, however, becomes weaker fairly quickly, revealing a more persistent tonic background (which can be seen more clearly in Fig. 1, c). At stimulation frequencies of 70, 80 and 100 per second the latent period of the tetanic response becomes longer; the amplitude and duration of the response show a decrease. There is simultaneous decrease in the tonic component of the contraction which becomes a small wave with the tetanic peak superimposed on it. Reflex tetanus disappears completely at 150 simulations per second, while the abortive tonic wave disappears only at 360 stimulations per second.

The more sluggish development of inhibition in the centers of the tonic apparatus is also shown by the following. When the afferent nerves are stimulated at frequencies at which complete inhibition develops in the centers of the fatigued sartorius muscle, no such inhibition develops in the centers of the fatigued tonic bundle; the latter responds to such stimulation by weak, slow waves of contraction.

The data presented above provide evidence confirming the hypothesis of lower functional mobility of nerve centers associated with tonic contractile musculature. In fact pessimal inhibition in these centers occurs at lower frequencies of stimulation than in centers associated with phasically active systems. The slower development of complete inhibition in tonic centers is not contradictory to this hypothesis. A. A. Ukhtomskii [3] indicated that "the more labile the tissues dealt with the more marked is the significance of intervals and urgency of performance of the component functions. Where the intervals are large, the individual moments of their interaction are of much less critical significance. That is why typical humoral influences in the body and tonic systems show relatively greater inertia than tetanic innervation of the musculature. That is also

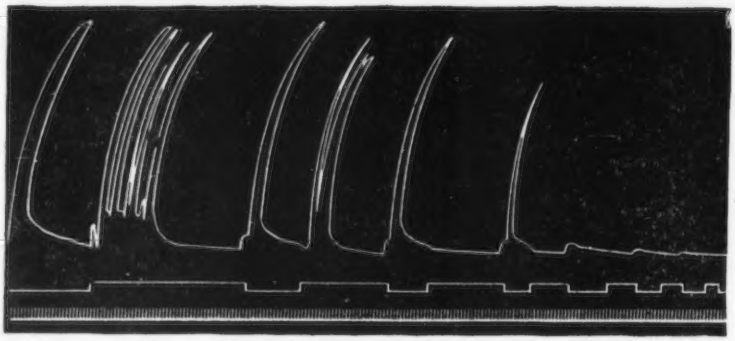

Fig. 2. Reflex contractions of iliofibular muscle. Stimulation of the central end of the sciatic nerve at the rates of 50, 70, 80, 100, 150, 220, 300 and 360 per second. Records the same as in Fig. 1.

why marked parabiotic stages presuppose a relatively high labile state of the experimental preparation." "Parabiotic inhibition" in the words of E. K. Zhukov [1], "is only possible in lively tissue with rapid metabolism and capacity for quick reaction."

SUMMARY

Experiments were performed on spinal frogs. It was demonstrated that in excessive increase of the frequency of stimulation of the afferent paths a condition of pessimal inhibition is developed in the nerve centers innervating the tonic muscular apparatus. Pessimal phenomena begin at the frequency of 60-70 stimulations per second. Complete depression of the reflex response takes place at the frequencies of 300-350 stimulations per second. As to the centers innervating the tetanic muscle fibers the pessimal phenomena commence only at the frequency of 100-150 per second. Inhibition in "tetanic centers" develops much quicker. Complete depression of the reflex response usually takes place at the frequencies of 150-200. These facts point to the lower lability of the nerve centers innervating the tonic muscular apparatus.

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THE EFFECT OF CERTAIN INHIBITORS OF CARBOHYDRATE-PHOSPHORUS METABOLISM ON THE TONIC AND TETANIC REACTIONS OF ISOLATED VERTEBRATE MUSCLE FIBERS

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The problem of tonus occupies a prominent position in modern physiology. Its physiologic mechanisms have at present received detailed study; studies of the biochemical processes associated with tonic contraction, however, have been fairly contradictory.

We have succeeded in suggesting certain differences in the carbohydrate-phosphorus metabolism supplying energy to tonic and to tetanic reactions based on our experiments on the action of inhibitors of carbohydrate-phosphorus metabolism in frog muscles [4, 5].

For further elucidation of this question work has been done on the isolated muscle fibers of frog and rat. It is known that frog muscle has three main types of muscle fibers: tetanic, tonic and transitional [1, 2]. We have recently established that similar types of muscle fibers can be isolated in rat muscle also.*

The present report deals with 170 experiments on tetanic, tonic and transitional fibers.

EXPERIMENTAL METHOD

The method for isolation of single muscle fibers in frog, their maintenance and recording of their contractions have been described in the work of Serkov, Sorokin, Zhukov, Leushina and other authors. The procedure for isolation of muscle fibers from rat muscles is reported by P. Honcke [6] and in a report by the present author.*

Stimulation of the fiber was effected by single induction shocks with voltage of 6 v in the primary circuit (accumulator). After the control contractions were recorded the Ringer's solution in which the muscle fiber was kept was replaced by solution of one or another inhibitor and stimulation of the fiber was carried out every 3-5 minutes to observe the character of contraction.

The following substances were used as inhibitors: 1) 2, 4-dinitrophenol, which abolishes respiratory phosphorylation and adenosine triphosphate and creatine phosphate breakdown; 2) sodium fluoride which interrupts glycolysis at the stage of pyruvic acid formation, and 3) sodium monoiodoacetate which inactivates the phosphoglyceraldehyde and dehydrogenase. It is characteristic for the action of this substance that no lactic acid is formed during contraction of the muscle.

EXPERIMENTAL RESULTS

a) Effect of Dinitrophenol

Solutions from 0.0025 to 0.00015 M were used. The majority of experiments was performed on transitional fibers which showed, on adequate stimulation, a typical tetanic peak and tonic plateau in control

* In press.

contractions. On weaker stimulation only rapid tetanic contraction of the fiber could be observed. Under the influence of dinitrophenol the tonus-like plateau gradually disappeared and the contraction became tetanic, evidently as the result of reduced excitability, since on stronger stimulation it was again possible to demonstrate the tonic component. Then the tetanic peaks disappeared and the response of the fiber to stimulation of a given strength ceased, but slow tonic contraction, which persisted for a fairly long time, could be demonstrated in response to stronger stimulation. 20-30 minutes after the disappearance of the tetanic peaks an increase in the latent period of tonic contraction and slowing of the development of contraction were noted; the latter's amplitude was reduced. After 40-60 minutes responses to all strengths of stimulation were lost (Fig. 1).

Tonic fibers usually exhibit similar changes in contraction as those shown by the tonic part of transitional fibers.

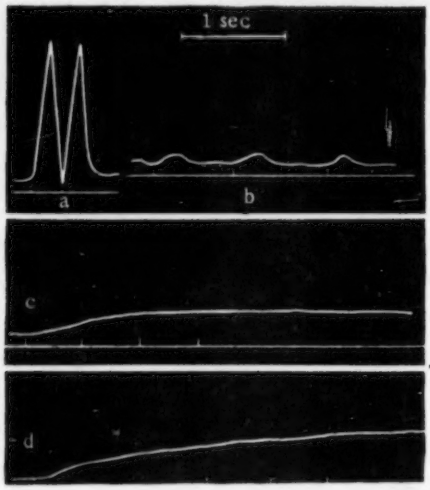

Fig. 1. Effect of 0.00125 M solution of 2,4-dinitrophenol on contraction of isolated transitional fiber of the ilio-tibial muscle in frog. a) Control contractions; b) contractions after 11 minutes of action of dinitrophenol (stimulation with induction current, distance between coils 25 cm); c) contractions after 31 minutes of dinitrophenol action; d) contractions after 41 minutes of dinitrophenol action (stimulation with induction current, distance between coils 8 cm).

Contractions of tonic fibers diminish and disappear under the influence of dinitrophenol with almost no change in character. The reaction of such fibers disappears much more rapidly than that of transitional and tonic fibers, but persists a little longer than that of the tetanic component in transitional fibers. With low concentrations of dinitrophenol (0.00015 M) tetanic muscle fibers maintain their contracting ability for a long time; the high-amplitude contraction observed in the control experiment decreases very rapidly (after 3-4 minutes); what remains is a small twitch of the fiber which may go on for hours.

Changes in rat muscle fiber contractions under the influence of dinitrophenol are similar to those described above. Rat transitional and tonic muscle fibers show particularly marked increase in the latent period of contraction under the action of dinitrophenol.

b) Effect of Sodium Fluoride

Sodium fluoride was used in concentrations from 0.01 to 0.03 M. Its action on frog transitional muscle fibers led to the rapid decrease and disappearance of tetanic peaks and development of tonic contraction in response to very strong stimulation, the contraction disappearing after a fairly rapid passage through the same phases as those seen under the influence of dinitrophenol.

Experiments with tonic fibers also showed gradual decrease and slowing of their contractions under the influence of sodium fluoride.

Contractions of tetanic fibers gradually diminished and disappeared. Sometimes it was possible to observe in the middle of an experiment that the response to stimulation began at the third-fourth impulse, the contraction being at first barely perceptible, then increasing gradually at the sixth-ninth impulse.

In rat transitional fibers the tetanic and tonic components disappear almost simultaneously under the influence of sodium fluoride. However, if the fiber is periodically immersed in pure Ringer-Locke solution for 0.5-1 minute after the disappearance of the tetanic component it is possible to achieve prolonged preservation of tonus-like contraction after cessation of tetanic peaks (Fig. 2).

Changes in the fast and slow fibers of rat under the influence of sodium fluoride were similar to those in the corresponding muscle fibers of frog.

c) Effect of Sodium Monoiodoacetate

Experiments were performed with 0.00003 M solutions of sodium monoiodoacetate.

As has been mentioned above, when a transitional fiber is stimulated by sufficiently strong current its contraction clearly shows a tonic background against which appear the tetanic peaks.

Under the influence of monoiodoacetate the described contraction of frog transitional fibers is so altered that first the tonic plateau becomes lower and disappears and then, very rapidly, disappearance of tetanic peaks occurs; these two phenomena sometimes take place simultaneously (Fig. 3).

Tonic fibers undergo the same changes in contraction under the influence of monoiodoacetate as those described for dinitrophenol and sodium fluoride.

Tetanic fibers show lower amplitude of contraction without change in its character under the influence of monoiodoacetate.

Monoiodoacetate elicits similar changes in rat muscle fibers. Development of irreversible contracture is characteristic for the action of monoiodoacetate.

E. Z. Moreva [3] suggests that the muscle's ability to function against the background of developing contracture elicited by monoiodoacetate may be explained by isolated muscle fibers being able to contract while they have not yet undergone contracture, whereas those fibers which had passed into a state of contracture cease to contract.

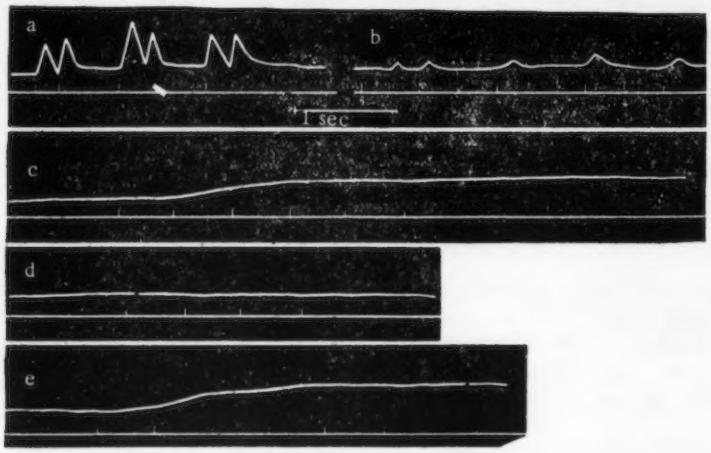

Fig. 2. Effect of 0.03 M solution of sodium fluoride on contractions of isolated transitional fiber of the heel muscle in rat. a) Control contractions (stimulation with inter-coil distance of 15 cm), b) contractions after 20 minutes of sodium fluoride solution action, c) contractions after 35 minutes of action of sodium fluoride solution, d) after 38 minutes of sodium fluoride action failure of the fiber to respond to stimulation, e) contractions of the fiber after being in Ringer-Locke solution for 1 minute (stimulation with induction current, distance between coils 10 cm).

Our data show that contractions can take place in the isolated muscle fiber even against the background of more or less pronounced contracture.

It is usually possible to restore the muscle fiber, frog or rat, from a state of complete cessation of contraction produced by one or other inhibitor to a state approaching the normal by immersing it for some time in Ringer-Locke solution. However, following the action of monoiodoacetate no restoration of muscle fiber contractility was ever observed.

The material presented suggests the following.

Dinitrophenol, which extinguishes respiratory phosphorylation, affects first of all tetanic contraction, while blocking of glycolysis, particularly blocking of lactic acid formation by monoiodoacetate, elicits first of all impairment of tonic reactions of muscle fibers.

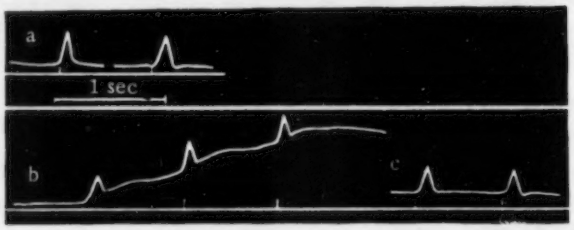

Fig. 3. Effect of 0.0003 M solution of sodium monoiodoacetate on contraction of isolated transitional fiber of ilio-tibial muscle in frog. a) Contractions on stimulation with induction current, distance between coils at 25 cm, b) contractions on stimulation with induction current, distance between coils 10 cm; c) contractions on similar stimulation after 50 minutes' exposure to monoiodoacetate.

Inhibition of metabolism by one or other inhibitor invariably leads to lowering of the amplitude of contractions without change in their character in the case of tetanic fibers, and a marked change in character as well as lowering of amplitude in the case of tonic fiber contractions: there is an increase in the latent period of contraction, an increase in the period of development and growth of contraction, slowing of post-contraction relaxation of the fiber, etc.

SUMMARY

Experiments on the influence of certain inhibitors of the carbohydrate-phosphorus metabolism on the contraction of various types of muscular fibers permitted the conclusion that the tonic and tetanic reactions change in a different way. Evidently, certain biochemical processes are especially important for tonus, while others for tetanus. Block by the inhibitors was reversible in character. No pronounced difference was found in the action of toxins on the muscle fibers of frogs and rats.

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*In Russian.

THE EFFECT OF STIMULATION OF THE HYPOTHALAMUS ON THE SECRETORY FUNCTION OF THE STOMACH IN LONG-TERM EXPERIMENT

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The number of references to the effect of hypothalamic stimulation on the secretory function of the stomach is very small. Such investigations have been carried out by J. Beattie [2] who found in short-term experiments on cats under barbiturate anesthesia that faradic stimulation of the lateral wall of the hypophysial infundibulum elicited secretion of gastric juice. T. Heslop [3] has shown that stimulation of the anterior hypothalamus in cats and dogs increases the secretion of gastric juice with a rise in acidity and higher content of free HCl. Similar changes in gastric secretion have been observed on stimulation of the vagus. Stimulation of the posterior part of the hypothalamus increased the amount of mucus secreted, and, in the presence of acid gastric juice, diminished its acidity. Similar reactions were observed on stimulation of the splanchnic nerve.

D. Sheehan [5] also found that stimulation of the posterior and especially of the lateral part of the hypothalamus led to considerable increase in the secretion of gastric mucus, accompanied by a series of sympathetic effects; this suggests the possibility of the existence of a sympathetic center in the hypothalamus.

There is much less data available on the increase of gastric juice acidity on stimulation of the anterior part of the hypothalamus. R. Porter, H. Movius and J. French [4] studied the effect of the hypothalamus on HCl secretion in short-term experiments on monkeys under cyclopropane anesthesia. Stimulation of the anterior part of the hypothalamus gave an HCl secretion curve similar to that obtained on stimulation of the vagus. Bilateral vagotomy abolished completely the effect of anterior hypothalamic stimulation and diminished the reactions of stimulation of the posterior hypothalamus. Preliminary extirpation of the adrenals abolished entirely the effect of stimulation of the posterior hypothalamus without altering the results of stimulation of the anterior hypothalamus. When vagotomy and adrenal extirpation were combined, stimulation of both parts of the hypothalamus proved ineffective. On the basis of these results the authors have postulated a theory of the existence of two different pathways mediating the action of the hypothalamus on HCl secretion by the stomach: the first originates in the anterior hypothalamus and reaches the stomach by way of the vagus, the second pathway begins in the posterior hypothalamus and is mediated by the pituitary-adrenal system.

Literature data show that the hypothalamus plays an important role in the regulation of the secretory function of the stomach. However, this subject has been relatively little studied and the data obtained are contradictory. The majority of investigations have been carried out in short-term experiments.

The present work is concerned with the study of the effect of hypothalamic stimulation on the secretory function of the fasting stomach under new experimental conditions.

EXPERIMENTAL METHOD

Our experiments were performed under long-term conditions on dogs with fistulas of the fundal part of the stomach (Basov's method). The animals were subjected to experiments on the 6th-7th postoperative day.

The dogs were kept fasting for 18-20 hours prior to experiment, and were allowed to rest for a day after the experiment. Gastric juice was collected in a measuring cylinder with subsequent determination of total acidity and of digestive power according to Mett over a period of 24 hours. Gastric juice was collected every 5, 10, 15 and 30 minutes depending on the aim of the experiment. Free HCl and total acid content were determined by titration with 0.1 N solution of NaOH, using dimethylamidoazobenzene (0.5% alcoholic solution) and phenolphthalein (1% alcoholic solution) as indicators. Determinations were made on 1 to 10 ml gastric juice. Acidity of the latter was expressed in the number of milliliters of alkali calculated on 100 ml gastric juice.

Following careful investigation of the secretory function of the stomach and accumulation of sufficient data, the animals were subjected to the operation necessary for application of 4-polar electrodes to the hypothalamus. The technique of application of multipolar electrodes to the hypothalamus for long-term experiments on dogs involving approach to the hypothalamus from the base of the brain has been developed in collaboration with P. G. Bogach and described in previous reports [1, 2]. Hypothalamic stimulation was achieved by means of electric current from a sound generator of "GZ-1" type.

EXPERIMENTAL RESULTS

Secretory function of the stomach was investigated in 5 dogs, of which one served as control. Following formation of the gastric fistula the gastric content was regularly examined. It was possible to collect from 2 to 10 ml gastric contents, usually opalescent mucus, from each dog over a period of 4-5 hours. Free HCl was absent, total acidity was within the range 15-25, digestive power 0. No change in gastric secretion was observed following the procedure of application of 4-polar electrodes to the hypothalamus.

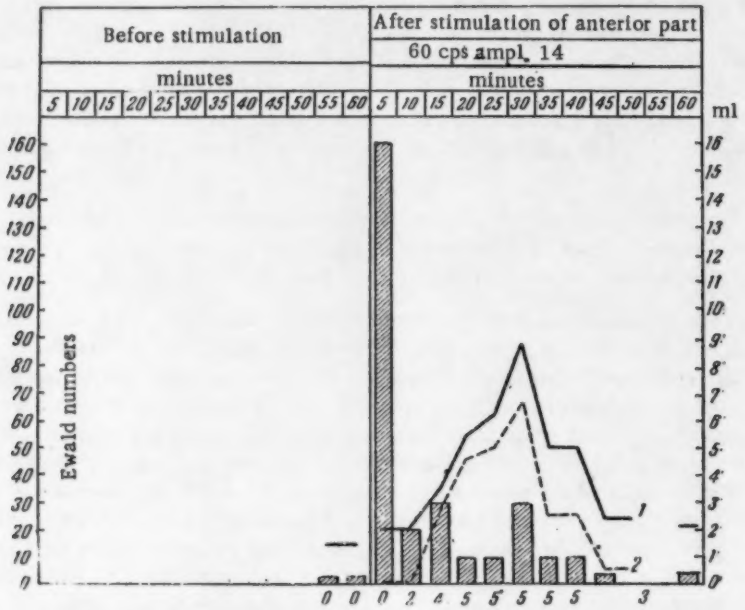

Fig. 1. Change in the gastric secretion following single stimulation of the anterior hypothalamus with electric current (frequency 60 cps, amplitude 14, duration of stimulation 1 minute). 1) Total acidity in Ewald numbers, 2) free HCl; columns show amount of gastric juice in milliliters for every 5 minutes, figures below – digestive power in millimeters of protein bar for 24 hours (Mett). Experiment No. 18, December 12, 1955, on dog Dzhek.

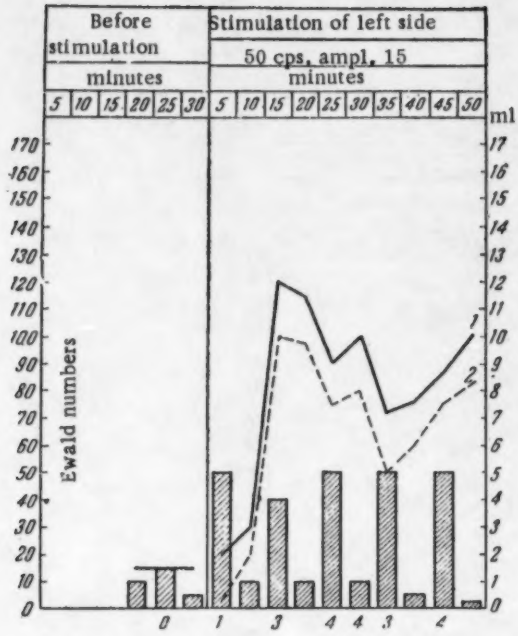

Fig. 2. Change in gastric secretion on stimulation of the left side of hypothalamus repeated every 10 minutes (electric current, 50 cps, amplitude 15, duration of stimulation 1 minute). Records the same as in Fig. 1. Experiment No. 20, December 16, 1955, on dog Dzhek.

Having satisfied ourselves that the operation of electrode application to the hypothalamus exerted no influence on the secretory function of the empty stomach, we began the experiments by investigating the gastric contents without the use of stimulation over a period of 1-2 hours and then started stimulating various areas of the hypothalamic region. Investigations showed that on stimulation of hypothalamic areas lying at the level of the anterior and lateral borders of the hypophysial infundibulum large amounts of gastric juice were secreted (from 20 to 150 ml), with high free HCl values (from 80 to 120), total acidity of 100-140 and digestive power of 3-7 mm.

Single stimulation was immediately followed by secretion of a large amount of gastric juice (up to 16 ml); the rate of secretion then gradually declined and secretion ceased after 50-60 minutes. Throughout the experiment (2-4 hours) single stimulation was associated with secretion of 20 to 30 ml gastric juice. The acidity of gastric juice following single stimulation reached a maximum at the 20-35th minute. The acidity decreased by the end of the experiment. The activity of gastric juice underwent changes parallel to the acidity.

Experiment No. 18, December 12, 1955, on the dog Dzhek can serve as illustration of the above (Fig. 1).

Multiple stimulation every 5, 10, 15 and 30 minutes of the same hypothalamic areas was associated with gastric secretion which was maintained at a high level throughout. During 3-4 hours of experiment from 40 to 150 ml gastric juice was secreted. Gastric juice acidity, as in experiments with single stimulation, reached a maximum after 20-30 minutes from the beginning of stimulation, but then remained high, with small fluctuations, until the end of the experiment. Similar changes were seen in the digestive power of the gastric juice.

Figure 2 shows the data obtained in experiment No. 20, December 16, 1955, in which the dog Dzhek was subjected to stimulation of the left side of the hypothalamus repeated every 10 minutes. The digestive power of gastric juice under these experimental conditions remained high throughout the experiment, whereas on single stimulation it decreased toward the end of the experiment.

Stimulation of the hypothalamic region lying at the level of the posterior border of the hypophysial infundibulum, both single and repeated, did not produce secretion of acid, active gastric juice; there was only slight increase in secretion of mucus without a rise in acidity and digestive power. This is well illustrated in Fig. 3 which shows the data for experiment No. 7, January 25, 1956, performed on the dog Grem.

We have used in our experiments a new method of isolated stimulation of the hypothalamus which permits precise localization of the area stimulated, whereas other authors carried out experiments under short-term conditions, using buried electrodes which have a number of disadvantages.

The findings of these authors are very incomplete, since they only noted an increase in acidity (Porter, Movius and French) or a rise in acidity accompanied by changes in the rate of gastric juice secretion (Heslop).

Our investigations were carried out under long-term conditions which permitted detailed study over a period of many months of all aspects of change in the secretory function of the empty stomach following stimulation of the hypothalamus. In addition to quantitative and qualitative examination of gastric juice we also

studied the character of changes in gastric secretion associated with single and repeated stimulation of the hypothalamus.

Moreover a careful study was made of the character of change undergone by the digestive power of gastric juice.

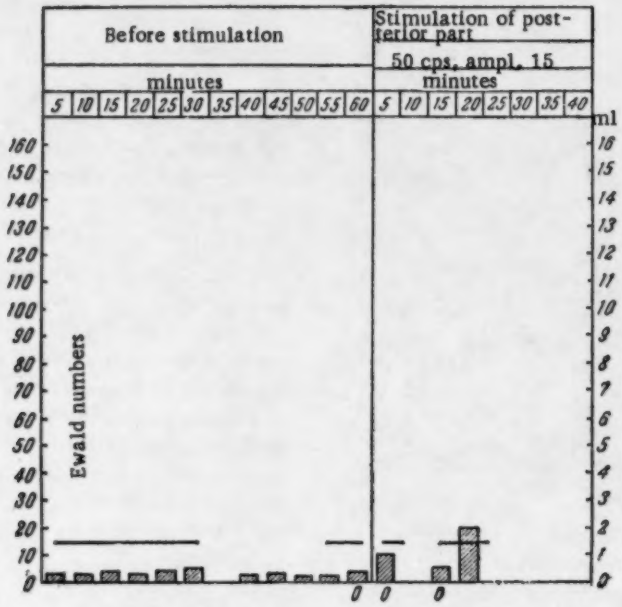

Fig. 3. Change in gastric secretion after single stimulation of posterior part of hypothalamus by electric current (frequency 50 cps, amplitude 15, duration of stimulation 1 minute). Records the same as in Fig. 1. Experiment No. 7, January 25, 1956, on dog Grem.

The results of our investigations suggest that stimulation of the anterior part of the hypothalamus produces a predominantly parasympathetic effect while stimulation of that part of the hypothalamus which is situated at the level of the posterior border of the hypophysial infundibulum gives a sympathetic effect. Stimulation of the anterior part of the hypothalamus leads to secretion of large amounts of active gastric juice with high acidity (predominantly parasympathetic effect), whereas stimulation of the hypothalamus at the level of the posterior border of the hypophysial infundibulum produces secretion of small amounts of gastric mucus (sympathetic effect).

SUMMARY

The effect of electrical stimulation of the hypothalamus on the secretory function of the stomach was studied in long-term experiments on dogs. It was revealed that stimulation of the anterior part of the hypothalamus during the period when there is no digestion causes secretion of large quantities of gastric juice with high acidity and digestive power. Stimulation of the hypothalamus with the level of the posterior edge of the infundibulum of the hypophysis gives a negligible increase of mucus secretion without the rise of acidity or of the digestive power.

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THE DEPENDENCE OF THE EFFECT OF STIMULUS STRENGTH IN ACID-DEFENSE CONDITIONED REFLEXES

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The law of dependence of the conditioned reflex effect on the strength of the conditioned stimulus has been established chiefly on the evidence of food conditioned reflexes. Manifestations of this law have subsequently been demonstrated in other reflexes as well, both in man and animals.

According to some literature data, however, acid-defense conditioned reflexes do not show such consistent dependence of effect on stimulus strength [6, 2].

Thus, V. K. Fedorov's experiments [6] on a dog in which food and acid-defense conditioned reflexes were established simultaneously showed that the food reflexes exhibited clear dependence of effect on stimulus strength while in the acid reflexes this dependence was constantly disturbed.

In the author's opinion this difference is determined by the fact that the acid unconditioned center, unlike the food one, does not possess a constant level of excitability.

This question received no further investigation. At the same time the offered explanation is in many respects inadequate, particularly with regard to the reason for the fluctuation in the level of excitability of the acid center. This problem therefore needs to be examined further.

EXPERIMENTAL METHOD

Experiments were carried out on two dogs. One of these (Belyi) had strong, balanced and mobile nervous processes, the other (Chernyi) belonged to the strong excitable type of nervous system. Two independent systems of conditioned reflexes were established in these animals – food and acid-defensive. Experiments with food reflexes were performed in the morning , those with acid reflexes in the evening . The following conditioned food stimuli were used: bell (strong) metronome 60 (medium), tone (weak); acid-defense reflexes were developed to a buzzer (strong), crackling noise (medium) and light (weak). Acid reflexes were reinforced by 5 ml 0.25% hydrochloric acid. The interval between the conditioned stimuli was 4 minutes.

EXPERIMENTAL RESULTS

Conditioned reflexes, both food and acid, were formed at approximately the same rate, within range of 10-15 combinations.

Food conditioned reflexes quickly reached a very high and stable level. The dependence of effect on stimulus strength was well marked.

In contrast to this, acid-defense conditioned reflexes were irregular both in magnitude and in length of the latent period and showed no consistent dependence of effect on stimulus strength (records of experiments No. 26 and 28 and No. 25 and 27 and Fig. 1).

Records

Belyi — Experiment No. 26 — 10/8/1954

Food reflexes

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
56	Bell	15	3	29	175	
47	Metronome 60	15	2	27	186	
45	Tone	15	4	23	165	
48	Metronome 60	15	3	25	160	
46	Tone	15	3	20	170	
57	Bell	15	2	22	155	

Belyi — Experiment No. 28 — 10/8/1954

Acid reflexes

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
61	Buzzer	15	7	12	50	
53	Crackling noise	15	5	15	64	
50	Light	15	9	9	105	
54	Crackling noise	15	13	5	70	
51	Light	15	5	15	65	
62	Buzzer	15	4	16	65	

Chernyi — Experiment No. 25 — 10/9/1954

Food reflexes

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
54	Bell	15	2	33	180	
45	Metronome 60	15	3	24	190	
42	Tone	15	2	25	175	
46	Metronome 60	15	2	29	195	
43	Tone	15	4	22	185	
55	Bell	15	2	30	180	

As can be seen from the records and Fig. 1, the magnitude of acid conditioned reflexes fluctuates greatly not only from experiment to experiment but also within a single experiment. Considerable variations in the secretory effect are also observed in response to unconditioned stimulation. Similar dynamics of acid-defense conditioned reflexes have also been described by other authors [2, 6].

Chernyi Experiment No. 27 10/9/1954

Acid reflexes

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
57	Buzzer	15	8	9	95	
51	Crackling noise	15	4	16	80	
49	Light	15	5	18	50	
52	Crackling noise	15	9	6	65	
50	Light	15	10	7	70	
58	Buzzer	15	9	3	90	

Belyi Experiment No. 32 10/14/1954

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
69	Buzzer	15	2	40	205	
61	Crackling noise	15	1	43	190	
58	Light	15	2	30	190	
62	Crackling noise	15	2	38	200	
59	Light	15	2	34	180	
70	Buzzer	15	1	45	195	

Chernyi Experiment No. 33 10/16/1954

No. of stimulus	Type of stimulus	Time of isolated action of conditioned stimulus in seconds	Latent period of conditioned reflex in seconds	Magnitude of conditioned reflex in scale divisions	Magnitude of unconditioned reflex in scale divisions	Remarks
69	Buzzer	15	1	53	195	
63	Crackling noise	15	2	44	210	
61	Light	15	2	41	200	
64	Crackling noise	15	1	48	195	
62	Light	15	2	37	205	
70	Buzzer	15	1	50	200	

The described phenomena can be understood if, in accordance with the factual material, it is assumed that the excitability of the acid center changes not only from experiment to experiment but also from combination to combination, with no directional consistency. However, there is no adequate evidence on the strength of which such physiologic properties could be ascribed to the acid center. The cause of these distinctive features of acid conditioned reflexes must, therefore, be sought elsewhere.

It has long been established that saliva is secreted predominantly from that salivary gland which is ipsilateral to stimulation of the oral cavity [1, 3, 4, 5]. This was particularly clearly demonstrated in K. S. Abuladze's experiments [1] on dogs in which two small symmetrical areas of the tongue were externalized together with the ducts of the two parotid glands.

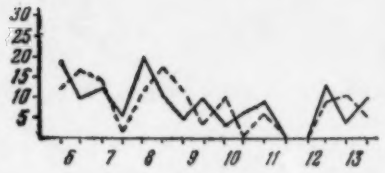

Fig. 1. Dynamics of acid-defense conditioned reflexes in the dog Belyi in response to a buzzer (solid line) and to light (broken line). Ordinate – amount of conditioned secretion in scale divisions, abscissa – number of experiments.

In work on acid-defense reflexes, however, the apparatus for introduction of the acid and the balloon for recording salivation are usually attached to the opposite sides of the dog's mouth for purposes of convenience. Irrigation of the oral mucosa with acid thus occurs chiefly on one side, while the salivary reaction is recorded from the gland situated on the opposite side, which is naturally exposed to much smaller amounts of acid.

It is natural to suppose that the peculiarities in the course of acid-defense conditioned reflexes were determined by the fact that stimulation was carried out on one side whereas salivation was recorded from the other.

This was fully confirmed by experiments. When salivary secretion was recorded from the side ipsilateral to stimulation in the same animals as those used in above-mentioned experiments, the unconditioned and conditioned reflexes showed a marked increase from the first day of experiment. From the third experiment using the new technique the conditioned reflexes became established at a new and much higher level (records of experiments No. 32 and 33).

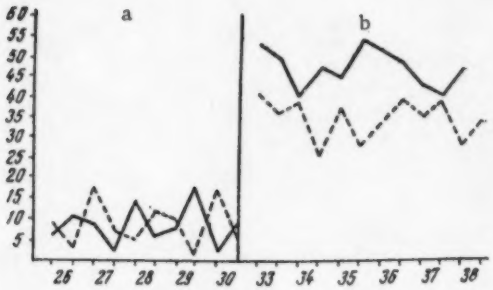

Fig. 2. Acid-defense conditioned reflexes in the dog Chernyi: a) on recording salivation from left parotid gland when acid is introduced from the right side of mouth; b) on introduction of acid and recording of salivation from the same side. Records as in Fig. 1.

The records show that the conditioned reflexes more than doubled on average, their latent period shortened to 1-3 seconds, they became stable and, most important, began to show consistent strength-effect dependence (Fig. 2). Moreover, while before food conditioned reflexes in these animals exceeded appreciably the acid-defense ones, the latter now became considerably greater than the former. This investigation provides, to our mind, convincing evidence that the absence of regular dependence of effect on stimulus strength is not a specific property of acid conditioned reflexes. There is also no basis for considering that the acid center, unlike the food center, is unable to maintain a given level of excitability.

SUMMARY

The law of dependence of the magnitude of reflexes on the strength of the stimulus is not less pronounced in acid-defense conditioned reflexes than in food reflexes.

The absence of consistent dependence of the effect on the strength of the stimulus noted by other authors was due to the peculiarities of the methods employed by them. If in formation of acid reflexes the acid is poured into the mouth of the animal on one side, while the salivary secretion is recorded on the other the conditioned reflexes show instability and the effect does not depend on the strength of the stimulus. This does not occur when the salivary secretion is recorded for the gland on the side of acid introduction.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE ROLE OF THE CEREBRAL CORTEX IN THE PATHOGENESIS OF HEMOLYTIC ANEMIA

COMMUNICATION II. THE EFFECT OF ACUTE DISTURBANCE OF HIGHER NERVOUS ACTIVITY ON THE COURSE OF EXPERIMENTAL PHENYLHYDRAZINE-INDUCED ANEMIA

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Various methods are available for the study of the role of the central nervous system in the regulation of various bodily functions under normal and pathologic conditions. Among such methods, one that has found extensive application is that of organic impairment or functional disturbance of one or other link in the nervous system, with subsequent observation of change in function or structure of the organ or system of organs under consideration [2-4, 8-10].

Investigation of the effect of disturbance of higher nervous activity on the course of phenylhydrazine-induced anemia, as well as reproduction of the phenylhydrazine anemia syndrome by way of conditioned reflexes, is among the more natural methods which make it possible to study the role of the cerebral cortex in the pathogenesis of experimental hemolytic anemia. If it were found that the usual reaction of the blood system to the introduction of phenylhydrazine into the body followed a different course in animals with disturbed higher nervous activity than in animals which were simply given phenylhydrazine, it would provide weighty evidence for participation of central mechanisms in the pathogenesis of the developing hemolytic anemia. The present investigation was undertaken with this hypothesis in view.

EXPERIMENTAL METHOD

Four series of experiments on 27 sexually mature white rats were carried out. Duration of each experiment – from 18 to 32 days. All the animals were maintained under uniform conditions of environment and diet.

In the 1st series of experiments (rats No. 1-5) a study was made of the spontaneous changes in red blood cells in white rats; in the 2nd series (rats No. 6-15) the course of anemia induced by administration of phenylhydrazine was followed; in animals of the 3rd series (rats No. 16-25) the course of phenylhydrazine-induced anemia was studied against the background of disturbed higher nervous activity and in the 4th series of experiments (rats No. 26-35) a study was made of the effect of disturbed higher nervous activity on the blood picture.

Disturbance of higher nervous activity was induced and maintained throughout the experiment. For this purpose daily use was made of extremely strong tactile-pain and auditory stimuli (current 40 v for 10-15 seconds with short intervals over a period of 3-10 minutes, accompanied by loud ringing), and the significance of conditioned stimuli in the stereotype pattern was altered.

The state of the higher nervous activity in animals of the 3rd and 4th series was judged by establishing a conditioned reflex stereotype prior to the beginning of trauma to the higher nervous activity; the stereotype consisted of two positive conditioned reflexes and differentiation with respect to them, established on the basis of an unconditioned tactile-pain stimulus – weak electric current.

On termination of observations on animals of the 4th series the brain of rats No. 30, 32, 33 and of 2 intact animals was examined microscopically for gross structural changes (necrosis, hemorrhage, lesions of the vascular wall). The appearance of such lesions is not excluded in cases in which electric current is used as unconditioned stimulus, and they could lead to the development of protective inhibition rebound in the cerebral cortex. The preparations were stained with hematoxylin-eosin. A total of 80 preparations from different parts of the brain was examined.

Phenylhydrazine was administered to the animals in a single dose of 0.02 g/kg subcutaneously, in the form of 1% aqueous solution. Before injection of phenylhydrazine the blood of the experimental animals was examined twice to determine the initial indices. The initial blood picture in animals of the 3rd and 4th series was determined after the establishment of the conditioned reflex stereotype before the beginning of trauma to the higher nervous activity, and after the development of a persistent neurotic state before administration of phenylhydrazine. Blood specimens were always taken from the experimental animals at the same time – after administration of phenylhydrazine; the number of erythrocytes, amount of hemoglobin, color index, and smear picture were determined.

EXPERIMENTAL RESULTS

Analysis of experimental data obtained from animals which were only given phenylhydrazine (series 2) shows that the number of erythrocytes in animals of this series reached minimal values on the 4th day after administration of the hemolytic poison (65.9%). As can be seen from the table, the hemoglobin content by this time also reaches the lowest figures (73.5%). On the 2nd day after administration of phenylhydrazine the peripheral blood shows all the signs characteristic for anemia: anisocytosis, macrocytosis, polychromatophilia and punctate basophilia of the erythrocytes. From the 4th day after phenylhydrazine injection, anemia development is replaced by intensive blood regeneration. The number of erythrocytes shows a progressive increase reaching 96.3% of the initial value by the 16th day. The increase in blood hemoglobin overtakes the increase in the number of erythrocytes and by this time reaches 102.6%. From the 9th day after injection of phenylhydrazine 4 out of 10 rats in this series of experiments show onset of normalization of the red cells, which is complete by the 13th day after phenylhydrazine administration. The color index throughout the period of observation is a little above 1; by the 6th day it reaches 1.17.

Animals of the 3rd and 4th series showed on the 8-10th day after the beginning of trauma to the higher nervous activity the development of persistent impairment of cortical dynamics, with predominance of inhibitory processes. As disturbances of higher nervous activity developed, positive conditioned reflexes disappeared, the animals' behavior underwent a change: the rats became untidy, sluggish, mostly sitting curled up with head tucked under; the coat lost its glossiness and smoothness and acquired a dirty yellow tinge.

In the case of the animals of the 3rd series, who were given phenylhydrazine after the development of a persistent neurotic state, the greatest drop in the number of erythrocytes and the amount of blood hemoglobin was also observed on the 4th day after injection of the hemolyzing agent. The number of erythrocytes by this time constituted 62% (see table) of the initial value, the amount of blood hemoglobin – 71.2%. Blood smears showed, on the 2nd day after phenylhydrazine injection, single polychromatophil erythrocytes in 7 out of 10 animals. On the 4th day of the development of anemia all the animals in this series showed marked anisocytosis, macrocytosis and large numbers of polychromatophilic erythrocytes. Blood regeneration which began at this time was much less intensive than in animals who were only given phenylhydrazine: on the 16th day after injection of phenylhydrazine the number of erythrocytes was on average only 76.2% and blood hemoglobin content 88% of the initial values. In 5 out of 10 animals the smears at this time showed all the changes characteristic for phenylhydrazine anemia. The color index was above 1 from the 4th day of anemia and remained within this range throughout the period of observation, reaching a maximum by the 9th day of anemia (1.26).

Under the influence of the disturbance of higher nervous activity, the animals of the 4th series showed development of moderate normochromic anemia which became more marked as the neurotic state became more profound (see table). The number of erythrocytes decreased gradually, amounting to 74.2% of the initial

value by the 16th day; the hemoglobin content was 73.7% of the initial value. No changes in the red cells could be seen in the blood smears apart from slight hypochromic tendency in the erythrocytes. The color index did not exceed 1 throughout the period of observation.

In animals of the 1st series (studies of red cell indices over a period of time corresponding to the duration of observations on animals of the 2nd, 3rd and 4th series of experiments) fluctuations in the indices under consideration did not extend beyond the limits of the initial values throughout the whole period of time.

No gross structural changes were seen on microscopic examination of the brain of rats No. 30, 32 and 33; the inhibition observed in animals of the 3rd and 4th series can therefore be regarded as a result of functional overstrain of processes of excitation in the cerebral cortex, and the results of these experiments considered from this viewpoint.

As can be seen from the table, the rebound inhibition developed as the result of overstrain of excitation processes exerts a definite influence on the course of experimentally produced hemolytic anemia, affecting particularly the phase of regeneration. Following administration of similar doses of phenylhydrazine, the animals with impaired higher nervous activity showed somewhat greater reduction in the number of erythrocytes and the amount of hemoglobin than that seen in intact animals. The development and disappearance of the blood picture characteristic for phenylhydrazine anemia were delayed in animals with impaired higher nervous activity as compared with animals who were only given phenylhydrazine. The increase in erythrocytes and hemoglobin in animals with impaired cortical activity followed a monotonous course with little tendency to normalization.

Dynamics of Change in the Number of Erythrocytes, Amount of Hemoglobin and the Color Index in Animals of the 2nd, 3rd and 4th Series

Days of observation following phenylhydrazine injection	Erythrocytes (in % of initial value)			Hemoglobin (in % of initial value)			Color index (absolute values)		
	series			series			series		
	2nd	3rd	4th	2nd	3rd	4th	2nd	3rd	4th
2nd	76,3	76,6	91,6	82,6	77,8	93,2	1,08	1,0	0,98
4th	65,9	62,0	87,8	73,5	71,2	93,0	1,06	1,18	1,0
6 th	69,7	65,8	90,8	84,4	75,8	93,5	1,17	1,2	1,0
9 th	83,6	68,1	86,1	92,4	80,6	82,2	1,09	1,26	0,9
13 th	91,5	75,3	81,8	94,4	88,0	77,1	1,03	1,2	0,9
16 th	96,3	76,2	74,2	102,6	88,0	73,7	1,03	1,1	1,0

Note: Figures cited are mean of 10 observations.

We tend to regard the development of normochromic anemia in animals of the 4th series as the result of profound impairment of trophic processes in the organism, including the hemopoietic tissue, consequent on disorganization of cortical activity. Our viewpoint finds confirmation in the work of T. V. Fokina [11] who observed increased hemoglobin breakdown and diminution of regeneration of red blood elements in experimental animals as the neurotic state became more profound. B. I. Balandurov [3] and N. S. Dzhavadian [6] observed the development of "spontaneous" normochromic anemia, together with a number of trophic changes, in animals deprived of normal cortical stimuli (decortication, transection of the spinal cord). D. I. Gol'dberg [5] noted that animals deprived of their cerebral cortices showed a more severe course of phenylhydrazine anemia and delay in restoration of blood indices as compared with animals who were only given phenylhydrazine.

The results of our experiments agree with the experimental material presented in the work of M. A. Usievich et al., [9, 10] who conclude that the "inhibitory state of the cortical cells leads to exceedingly low levels of activity of the systems studied." Our data also coincide with the experimental results of V. N. Chernigovskii and A. Ia. Iaroshevskii [12], E. L. Kan and V. N. Chernigovskii [7] and G. I. Alekseev [1] who observed inhibition of erythropoiesis in animals with inhibited cortical activity.

The results of our investigations thus confirm the data presented in the literature concerning the role of cortical mechanisms in the pathogenesis of experimentally induced hemolytic anemia [5] and indicate the dependence of the erythropoietic function of bone marrow on the functional state of the cerebral cortex.

SUMMARY

The author studied the effect of acute disturbance of the higher nervous activity, caused by overstrain of the processes of excitation in the cerebral cortex, on the course of experimental hemolytic (phenylhydrazine) anemia. In animals with disturbed dynamics of the cortical function the course of anemia after administration of the same doses of hemolytic toxin was much more severe than in intact animals. With further development of disturbance of higher nervous activity the processes of blood regeneration became less intense. Moderate normochromic anemia develops in intact animals under the influence of disturbance of higher nervous activity. This anemia progresses with further development of disturbances of cortical dynamics. The results of these experiments demonstrate the role of cortical mechanisms in the pathogenesis of phenylhydrazine anemia. They confirm the literature data on the dependence of erythropoietic function of bone marrow on the functional condition of the cerebral cortex.

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THE EFFECT OF INTRAVENOUS NOVOCAIN INJECTIONS ON THE BLOOD SYSTEM

COMMUNICATION II. CHANGES IN COMPOSITION OF THE BLOOD AFTER A SINGLE INJECTION OF NOVOCAIN

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An earlier report [11] was concerned with changes in the composition of the blood in rabbits and dogs resulting from repeated intravenous injections of 4-5 ml 1% solution of novocain . The present report presents data on the reaction of the blood system during the first few hours following injection of novocain .

EXPERIMENTAL METHOD

The experimental conditions and division of the animals into groups were the same as in the previous instance (see Communication I). Experiments were performed on 30 rabbits and 2 dogs. Blood was examined ½, 1, 2, 3, and 4 hours after injection of novocain solution.

EXPERIMENTAL RESULTS

Leukocyte reaction. Changes in the number of leukocytes which arise in response to novocain injection are fairly consistent in character (Fig. 1 and 2) and consist of an initial drop in their number (in 22 out of 30 animals) with subsequent increase (in 27 cases). The increase in the number of leukocytes varied between 15.1 and 346.1% of the initial level or (in absolute figures) between 1000 and 20,645 cells per 1 mm^3. Control observations showed that spontaneous fluctuations in the elements being studied could reach 69.1% and variations after injection of 4-5 ml physiologic solution 87.1% of the initial value. However, the characteristic curve described above with an increase in the number of leukocytes by the 2nd hour is only noted after injection of novocain . The reaction to novocaine was more marked than that to an injection of physiologic solution in 20 out of 30 rabbits and in both dogs, and in 10 animals the reverse was observed. In order to clarify whether repeated injection of novocain had any significance special investigations were undertaken; it was found that only in 12 out of 30 rabbits the maximal effect was observed after the first injection (unlike the previously established effect of novocain on conditioned reflex activity and arterial blood pressure). The increase in the number of leukocytes was not usually accompanied by changes in the differential leukocyte count.

Experiments in which novocain was administered to animals with previously (20-40 days earlier) denervated carotid sinuses and spleen throw some light on the mechanism of the changes observed by us. The significance of normal innervation of these organs for the regulation of hemoglobin and erythrocyte content has been repeatedly confirmed in recent years [4-10, 14, 13].

At the same time the leukocyte reactions in cases of impaired innervation of the spleen and carotid sinuses have remained practically uninvestigated. Experimental results showed that in 12 out of 14 animals, beginning from the 2nd-3rd month postoperatively, the increase in the number of leukocytes in response to novocain injection rose sharply (Fig. 1, IV-VII) and this could be observed during the next 12-18 months. Sometimes (in 3 cases; see table) the phase of enhanced reaction was preceded by a period of reduced reaction.

A similar reaction was observed also in animals with denervated spleens. Normal carotid sinus and spleen innervation is by no means the only factor on which the leukocyte reaction depends. A significant role was shown to be played by blood-letting (25-30 ml blood) sustained 25-30 days earlier. Thus, in 11 animals the average figures for increase in the number of leukocytes after injection of novocain before blood-letting varied within the range of 15.1-346%, after blood-letting from 33.4-420.7%. Enhanced intensity of leukocyte reactions was noted in 7 out of 11 rabbits (Fig. 2, III-V).

After blood-letting, unlike the situation before it, the most marked reaction occurred in response to the first injection of novocain (in 9 out of 11 animals).

For example, in rabbit No. 21 in experiment dated May 22, 1957 the increase amounted to 101.8% of the initial, and in experiment dated May 30, 1957 it was 46.2%. In rabbit No. 22 in experiment of May 24, 1957 the increase was 839.4%, in experiment of June 1, 1957 – 237.5% and in experiment of June 7, 1957 – 185%; the corresponding figures in the same rabbit prior to blood-letting were 346 and 419.8%.

Changes in hemoglobin, erythrocyte and reticulocyte content. The view that these blood elements are relatively little subject to rapid fluctuations has become fairly firmly established in the literature.

In this connection investigations have recently appeared [12] in which changes in the number of erythrocytes and the amount of hemoglobin occurring 3-4 hours after administration of test substances serve as an indication of whether the latter contain hemopoietic or anemia-inducing agents.

Our investigations showed that injections of both physiologic solution and novocain could produce fluctuations in the numbers of erythrocytes in excess of spontaneous variations (Fig. 1, I); so-called spontaneous fluctuations in erythrocyte content, according to our data (repeat examinations performed on 30 rabbits) amounted to 0-15.9% of the initial, or from 0 to 600,000 erythrocytes per 1 mm^3. Reduction in the number of erythrocytes was noted within the limits of 4.8 to 16% of the initial, or from 100 to 620,000 erythrocytes per 1 mm^3 (the latter was only observed in one case).

Intravenous injection of physiologic solution (4-5 ml) can lead to decrease or increase in the number of erythrocytes (to 24.6% of initial, or to 1,290,000 per 1 mm^3 in the latter case, and to 35.2% of initial or 1,950,000 per 1 mm^3 in the former). Reduction in the number of erythrocytes was observed in 24 out of 30 animals, increase in their number in 16. Injection of novocain produced approximately the same reaction with the difference that reduction in the erythrocyte content was somewhat more pronounced than that seen after injection of physiologic solution to the same animals (in 21 out of 30 cases). Increase in the number of erythrocytes fluctuated (average figures) from 0 to 32%, decrease in their numbers varied from 1 to 23% of the initial.

In absolute figures variations in the number of erythrocytes constituted 90,000-1,400,000 cells (increase) and 40,000-1,240,000 (decrease).

We are thus led to state that injections of physiologic solution (if they are not "extinguished" by multiple repetition) and of novocain give rise to fairly marked changes in the number of erythrocytes during the first 4 hours after injection. This suggests that changes in the number of erythrocytes immediately following injection of any substance cannot serve as an indication of the presence in it of stimulators or inhibitors of erythropoiesis.

Denervation of the carotid sinuses affected the reaction under consideration in such a way that 1-2 months after the operation, when normalization of the blood composition had apparently taken place, injection of novocain produced, as a rule, no erythrocytosis, while the reduction in the number of erythrocytes was more marked. Sometimes this phenomenon lasted for 9 months postoperatively (rabbits No. 4 and 5). This altered reaction to novocaine may be related to the reflex character of erythrocytosis noted by M. L. Belenkii and Iu. N. Stroikov [2], to the facts concerning the role of the carotid sinuses in the onset of erythrocytosis during hypoxemia established by N. N. Beller [3] and to the work of W. Grant [12] and M. Terzioglu [16].

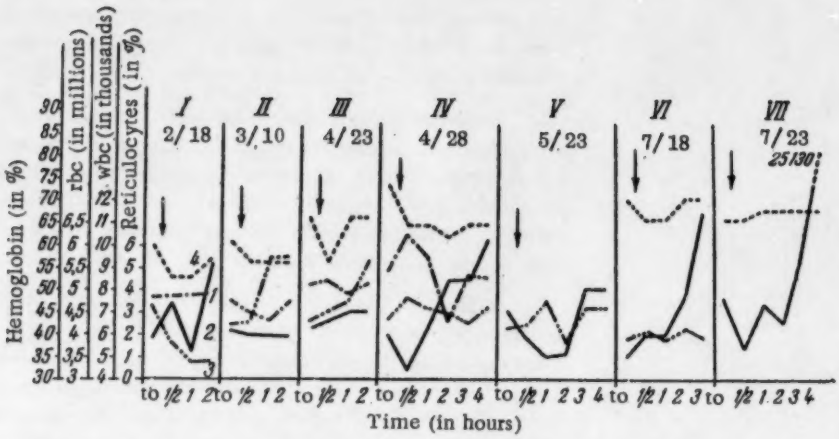

Fig. 1. Changes in composition of the blood in rabbit during 4 hours after intravenous injection of 5 ml 1% solution of novocain . I) Before denervation of carotid sinuses (denervation performed February 19, 1956); 1) erythrocyte content, 2) leukocyte content, 3) reticulocyte content, 4) hemoglobin content ↓) novocain injection.

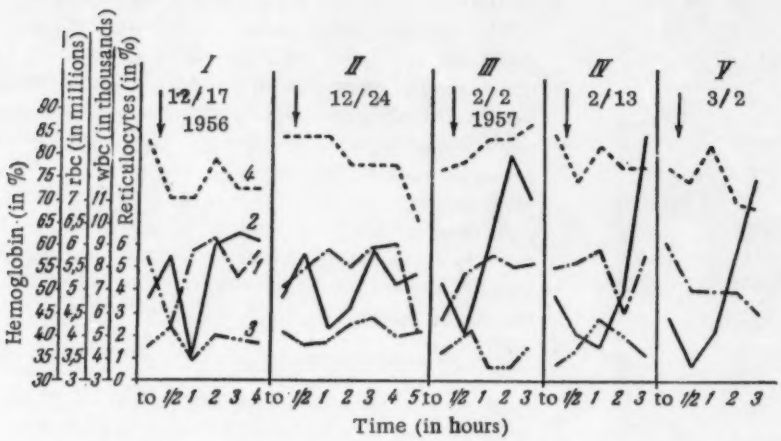

Fig. 2. Changes in composition of the blood in rabbits during 4 hours after intravenous injection of 5 ml 1% solution of novocain . I-II) Before blood-letting, III-V) after blood-letting (blood-letting January 9, 1957, 20 ml blood). Records the same as in Fig. 1.

The effect of preceding blood-letting was reflected in that injection of novocain caused a more marked drop in the number of erythrocytes in 7 out of 11 animals than was observed prior to this procedure; at the same time less marked increase in the number of erythrocytes was also seen in 7 animals. Thus, rabbit No. 20 showed an average drop in the number of erythrocytes following injection of novocain without previous blood-letting amounting to 13.5%, and an increase by 7% (after 2 months); after blood-letting the corresponding figures were 30.6% and 6.7%.

Fluctuations in hemoglobin content in healthy animals following injection of novocain are not very large, but there is a predominant tendency toward its reduction (Fig. 1, I and Fig. 2, I). The increase, according to out data, amounts to +10 units, and the decrease to −16 units. We, like a number of other authors [1, 5, 14] observed in more than half the cases no parallelism in the course of changes in the number of erythrocytes and of hemoglobin content (in 16 out of 30 animals).

Changes in the Number of Leukocytes in Rabbit No. 5 on Injection of 1% Novocain Solution Before and After Carotid Sinus Denervation

1/5 1956		1/12 1956		1/16 and 1/31 1946. Denervation of carotid sinuses	3/18 1957		3/10 1957		4/24 1957		5/14 1957		6/5 1957		7/24 1957		8/10 1957		8/24 1957		9/27 1957	
A	B	A	B		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
+1200	21,1	+2800	+35,1		-1400	+17	0	0	0	0	+6000	+74	+6470	+71	+20 670	+198	+35 200	+379	+10 900	+119	+11 730	+130
—0	0	—720	—17,5		—0	0	—3340	—27	—6360	—48	0	0	0	0	0	0	0	0	—2700	—3,2	—2530	—30

Note: A) Absolute number in thousands per 1 cm^3, B) percentage of initial level.

When carotid sinuses were denervated the animals no longer (from the 30th-40th postoperative day) showed an increase in hemoglobin content, and in some cases a decrease in the latter was observed.

Studies of the reticulocyte content of peripheral blood and of bone marrow contribute toward the solution of the question concerning the mechanism of changes elicited in response to injection of novocain . It was found that novocain administration to healthy animals produced no increase in blood reticulocytes; fluctuations in their number were diphasic in character in 14 out of 30 animals.

In absolute figures the fluctuations were from 1 to 22 per 1000 erythrocytes. In 3 experiments a decrease in the number of reticulocytes (to 27 per 1000 erythrocytes by the 4th hour of investigation; Fig. 1, I) was marked. In 2 experiments it could still be seen that following a preceding injection of novocain the reticulocyte content remained high even after 5 days, amounting to 59 per 1000 (with an initial level of 21 per 1000 erythrocytes). Carotid sinus denervation is known to be accompanied during the first 3-4 weeks by marked reticulocytosis, which then usually disappears. However, if novocain is injected at this time it produces marked increase in the number of reticulocytes in the peripheral blood (Fig. 1, II, III).

Attempts to elicit such an increase in the number of reticulocytes in rabbits at a later period proved unsuccessful. It should be recalled that during this period the bone marrow [3, 4] shows inhibition of erythropoiesis and absence of enhanced reticulocytosis. It seems probable that in the period between the 2nd and 4th month after denervation the injection of novocain may encourage egress of reticulocytes from the bone marrow. As regards the effect of preceding blood-letting, injection of novocain after normalization of the composition of the blood and the number of reticulocytes had dropped, not only did not cause an increase in their number, but was associated with less marked reticulocytosis than prior to blood-letting (rabbits No. 29, 30 and 32; Fig. 2, III, V).

The experiments carried out permit the following conclusions:

1. Intravenous administration of 4-5 ml 1% novocain solution to rabbits elicits, in healthy animals, marked leukocytosis beginning with the 2nd hour after injection, with frequently a preceding phase (during the first 30-60 minutes) of reduction in the number of leukocytes. Carotid sinus and spleen denervation leads to marked enhancement of the leukocyte reaction under consideration beginning from the 2nd postoperative month and lasting up to 1-$1\frac{1}{2}$ years. Blood-letting also enhances this reaction.

2. Changes in erythrocyte and hemoglobin content during 4 hours after injection of novocain are not consistent but are fairly marked both in the direction of decrease and increase of these indices. Denervation of the carotid sinuses leads to the disappearance of erythrocytosis in response to novocain.

3. Healthy animals do not usually show a reticulocyte reaction to novocain injection. After denervation of carotid sinuses, when the reticulocyte content of bone marrow is raised, injection of novocaine elicits an appreciable increase in the number of reticulocytes in peripheral blood.

SUMMARY

The author studied the changes which took place in the composition of the blood within 4 hours following the injection of 4-5 ml of 1% novocain solution to 30 rabbits. The number of leukocytes was increased in healthy animals in 2 to 4 hours.

After denervation of carotid sinuses and spleen this reaction was considerably intensified (for 2-18 months after the operation). Blood-letting acts in the same way but its effect is shorter. Changes in the number of erythrocytes and in the hemoglobin content are not consistent, but are rather pronounced. In $^{2}/_{3}$ of all cases this response to injection of novocain was more marked than that due to injection of physiological saline. 3-4 weeks after denervation of carotid sinuses novocain injection caused an increase in the number of reticulocytes in the peripheral blood due to their egress from the bone marrow.

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THE EFFECT OF PNEUMOPERITONEUM ON THE INFLAMMATORY REACTION OF RABBIT'S PINNA TO THERMAL BURN

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Pneumoperitoneum is one of the measures used successfully in the treatment of a number of pathologic processes (tuberculosis of the peritoneum and the intestine, plumonary tuberculosis, specific and nonspecific lesions of the pelvic organs, peptic ulcer etc.).

There are many publications dealing chiefly with the therapeutic merit of penumoperitoneum [3, 5, 7, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 and others]. Experimental investigations of this question are considerably less in number [2, 4, 6, 12 and others].

We have shown in our work [8, 9, 10, 11] that as the result of application of pneumoperitoneum to animals distinct changes appear in the excitability of the vasomotor and respiratory centers, as well as changes in thermoregulation and qualitative leukocyte reactions. There are references in the literature to changes in the course of inflammatory reactions under the influence of air introduced into the abdominal cavity. S. S. Laguchev [6] found that the reactivity of connective tissue and epitheliumwas altered in animals who had air introduced into the abdominal cavity; this alteration manifested itself in accelerated healing of cutaneous wounds.

The present communication deals with the results of observations on the effect of pneumoperitoneum on inflammatory reaction.

EXPERIMENTAL METHOD

Inflammation of the rabbit pinna, induced by thermal burn, served as model of inflammatory reaction. The burn was produced by water heated to 53°, in which the rabbit pinna was immersed for 3 minutes. The experiments were so designed that control and experimental animals were matched for weight, color and sex. Inflammation was first induced in one pinna, then, after healing (20-30 days later) in other. Thus those rabbits which were subjected to pneumoperitoneum in the first instance served as controls in the second and vice versa. The same animals thus served for the main observations (application of pneumoperitoneum) and for control (without application of pneumoperitoneum).

The inflammatory reaction was recorded by the A. S. Saliamon (cited in [1]) method.

Inflammatory edema was determined by measuring the volume of the pinna before and during the course of the inflammatory process – 2, 5, 24, 48 hours and 5 days after the burn. The volume was measured by immersing the pinna in a glass cylinder with diameter of 4 cm, filled with water. The magnitude of edema was expressed in the increase of volume of the inflamed pinna in percentage of its initial volume prior to the burn. The temperature at the focus of inflammation was measured with a thermocouple with a copper-constantan junction.

In addition to the objective recording of changes in temperature and extent of edema of the inflamed

pinna as determined visually and photographically, the third manifestation of inflammation – impairment of function of the muscular apparatus of the pinna – was also determined.

A total of 18 experiments was carried out, of these 9 served as controls. The experimental rabbits were subjected to pneumoperitoneum prior to the infliction of thermal burn.

In 6 experiments from 100 to 150 ml air was introduced into the abdominal cavity 30 minutes before the burn; refills were made after one day, then after 2 days. The gas bubble was monitored by roentgenoscopy.

In 3 experiments air was also introduced 30 minutes prior to the burn but in larger volumes – from 300- to 400 ml without refills. Observations in these investigations extended over 5 days.

EXPERIMENTAL RESULTS

In experiments in which air was introduced into the abdominal cavity of rabbits in small volumes (100-150 ml) the inflammatory reaction developed less strongly and was much less marked than in control animals. Thus, 48 hours after introduction of air the mean increase in volume of the inflamed pinna with respect to its initial volume was in control experiments equal to 135% and in animals with pneumoperitoneum to 66%. Consequently, edema in experiments with pneumoperitoneum was half that observed in control experiments (Fig. 1).

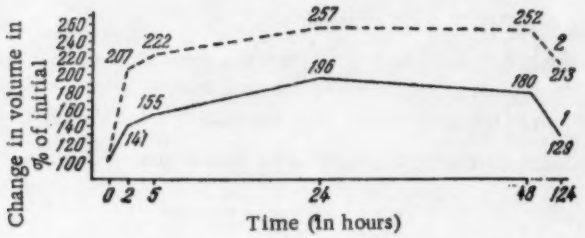

Fig. 1. Effect of pneumoperitoneum on change in volume of inflamed pinna in rabbit in percentage of the initial. 1) Experiment, 2) control.

The temperature of the inflamed pinna 48 hours after the burn was raised by an average of 3.8° in the control and by 2.3° in the experimental animal. The rise in temperature of the inflamed pinna in rabbits with pneumoperitoneum was thus 1.5° lower than in the control (Fig. 2).

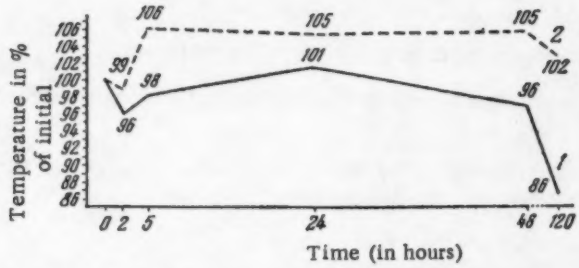

Fig. 2. Effect of pneumoperitoneum on changes in temperature of the inflamed pinna of rabbit in percentage of the initial. 1) Experiment, 2) control.

Definite differences between the experiment and control were also found with respect to the third index of inflammation, viz., impairment of the muscular apparatus of the pinna.

Unlike the results of experiments with introduction of a small amount of air, observations on cases in which a large amount of air (300-400 ml) was introduced into the abdominal cavity showed that the

inflammatory reaction was considerably more pronounced in the experimental rabbits compared with the controls. The mean increase in volume of the inflamed pinna in these experiments was 38% higher in animals with pneumoperitoneum than in the control ones.

No clear differences in the temperature of the pinna in experimental and control animals were observed.

Studies on the effect of pneumoperitoneum showed, as did our previous observations, that introduction of small volumes of air into the abdominal cavity exerted a normalizing effect on a number of physiologic mechanisms including those participating in the formation of inflammatory reaction. Relatively small volumes of introduced air inhibited the development of inflammation; its onset was delayed and the process was less pronounced than that in controls. The effect of large volumes of air introduced into the abdominal cavity proved to have an entirely opposite effect which had no anti-inflammatory influence.

With inflammation serving as a model we could, therefore, find confirmation of the duration and profound extent of the influence exerted by pneumoperitoneum. It is evident that the latter could be mediated by the reflex pathway as well as by way of change in other indices studied by us earlier. The data obtained confirm the view that the leading mechanism in the therapeutic effect of pneumoperitoneum on one or other pathologic process is by no means its purely mechanical action on the organism, but a neural mechanism, as has been repeatedly pointed out by Soviet authors [2, 3, 6-19 and others].

SUMMARY

Pneumoperitoneum of small volume (100-150 ml) exerts a normalizing effect on a number of physiological mechanisms controlled by the nervous system and involved in the reaction of recovery. Large volumes of air (300 to 400 ml) injected into the abdominal cavity have no anti-inflammatory effect.

The mean effect of pneumoperitoneum is mediated by the nervous mechanism.

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CHANGES IN THE SECRETORY AND MOTOR FUNCTION OF THE STOMACH IN EXPERIMENTALLY INDUCED GASTRITIS

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The use of surface coagulation of the gastric mucosa by 10% solution of $AgNO_3$ for production of inflammatory changes accompanied by inhibition of gastric secretion was developed in the laboratory of I. P. Pavlov [1, 4]. In recent times this method has been used for the study of blood and cardiovascular changes associated with gastritis [3, 5, own observations].

EXPERIMENTAL METHOD

In the course of the present work the secretory-motor function of the stomach was investigated in 2 dogs with gastric fistulas. Inflation of a rubber balloon introduced into the stomach was used as a stimulus. Gastric juice was collected every 15 minutes during the first hour of the experiment; each specimen was examined for amount of juice, total acidity and free hydrochloric acid. The motor activity of the stomach was recorded manometrically during a period of 2 hours; the gastric tonus was judged from the water manometer readings. The experiments were carried out 18-19 hours after the last meal. Experimental gastritis was produced by irrigation of the gastric mucosa, by way of the fistula, with 1000 ml 10% solution of $AgNO_3$ over a period of 5 minutes.

EXPERIMENTAL RESULTS

The character of gastric secretion of the dog Reks in response to mechanical stimulation under normal conditions and during experimentally induced inflammation of the mucosa is presented in the table and in Fig. 1.

Changes of Gastric Secretion in the Dog Reks Before and After Surface Coagulation of the Mucosa

Secretory indices	Before coagulation of the mucosa	After coagulation of the mucosa		
		1st week	2nd week	2nd month
Hourly output in ml	16—60	0	10—12	4—14
Free HCl in %	0,226—0,378	0	0—0,164	to 0,124
Latent period in minutes	5—10	0 (mucus in large quantities)	15—60	10—30

As can be seen from the table, during the first few days following surface coagulation there was no secretion; only considerable amounts of mucus, sometimes bloody, were observed. A prolonged period of hyposecretion set in from the second week, and lasted about 2 months. Gradual restoration of secretion in response to mechanical stimulation was only seen during the 3rd month after surface coagulation of the mucosa.

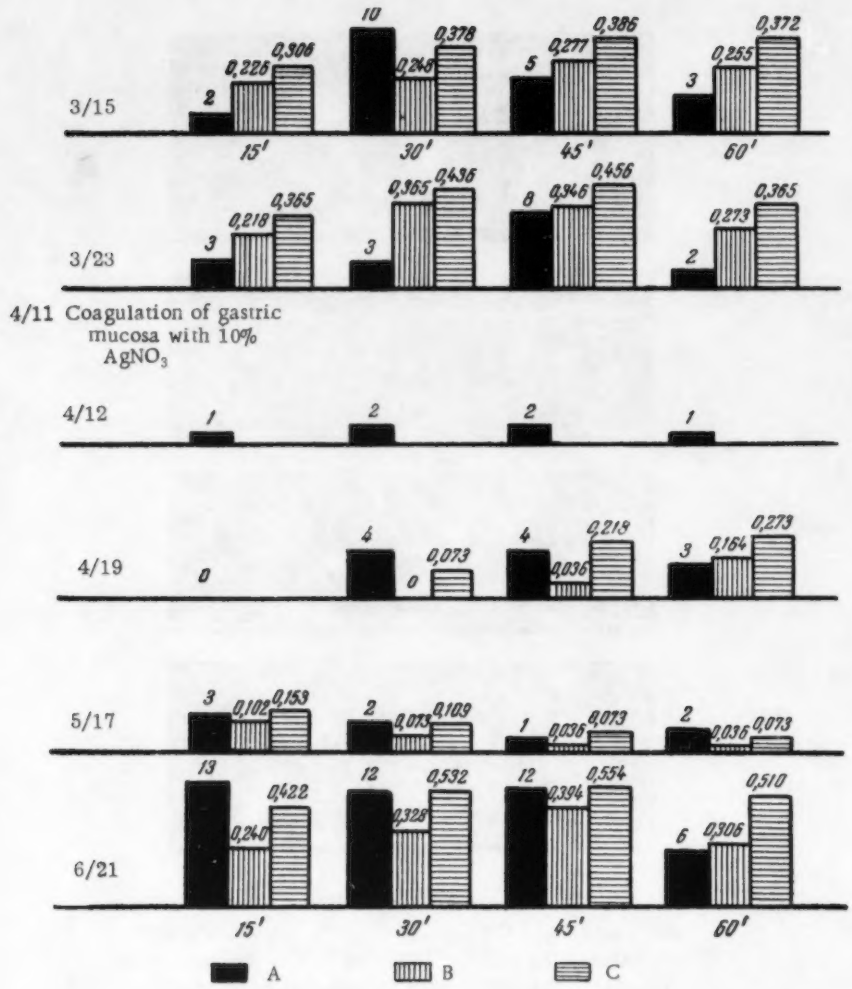

Fig. 1. Changes in gastric secretion in experimentally induced gastritis, dog Reks. A) Amount of gastric juice in milliliters; B) free hydrochloric acid in percentages; C) total acidity in percentages.

A similar sequence of changes was noted in the case of the dog Dichka with the difference that there was no complete inhibition of secretion during the first week.

In the normal state the motor activity of the stomach continued throughout the time of mechanical stimulation. The dog Reks showed rhythmic contractions of 10-12 seconds' duration 1-5 minutes after the onset of stimulation. By approximately the end of the first hour prolonged increase in gastric tonus (to 36-40 cm H_2O compared to the initial 20-24 cm), lasting 40-60 seconds with pauses up to 1 minute. This character of gastric movement was maintained until the end of recording. In the case of Dichka, the latent period was longer and contractions more frequent, seen against a background of moderately increased gastric tonus (from 24-26 to 30-32 cm H_2O). These contractions were only infrequently replaced by slower waves.

The day following surface coagulation of gastric mucosa both dogs showed profound inhibition of gastric motor activity, despite the same strength of mechanical stimulation. This occurred against the background of increased gastric tonus (30-32 H_2O). During subsequent days (first week) the motor activity increased gradually. Low amplitude waves with long intervals between them appeared after a prolonged latent period of 20-30 minutes.

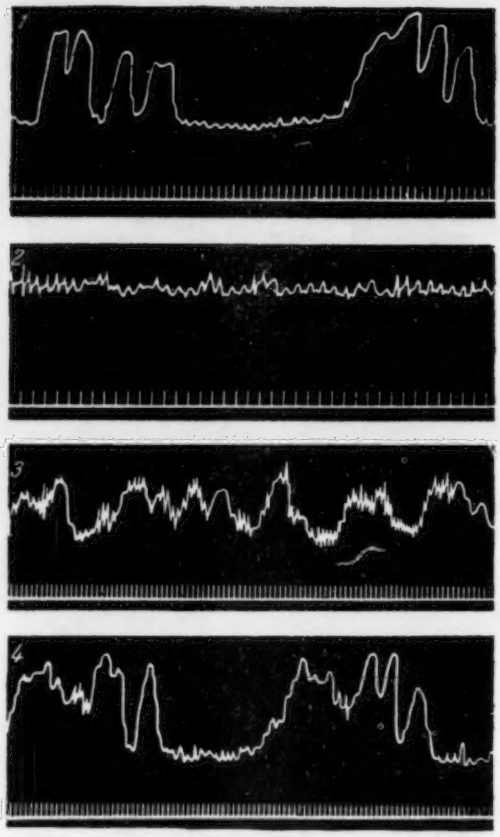

Fig. 2. Changes in motor activity of the stomach in experimental gastritis in the dog Reks. All the gastrograms recorded during the middle of the 2nd hour of the mechanical stimulation; 1) Normal motor activity, 2) 2nd day of gastritis, 3) 10th day of gastritis, 4) recovery of normal motor function 1 month after surface coagulation of mucosa. Time marker – 3 seconds.

This type of motor activity resembles "fasting periodicity." Seven-ten days after surface coagulation the motor activity assumed a haphazard character. This type of curve may be likened to the "arrhythmic" type described clinically in chronic gastritis [2].

The initial character of motor activity was restored in both dogs by the end of the first month after coagulation of the mucosa.

The dynamics of the described changes are presented in Figs. 2 and 3.

The damaging effect of an experimental pathogenic factor on the motor function of the stomach thus proved to be less marked and more transient than its effect on the secretory function.

The uniformity of secretory impairment, viz.,inhibition, indicates that the model of gastritis employed cannot reflect fully all the multiplicity and variety of disturbances of the secretory process in inflammatory diseases of the stomach.

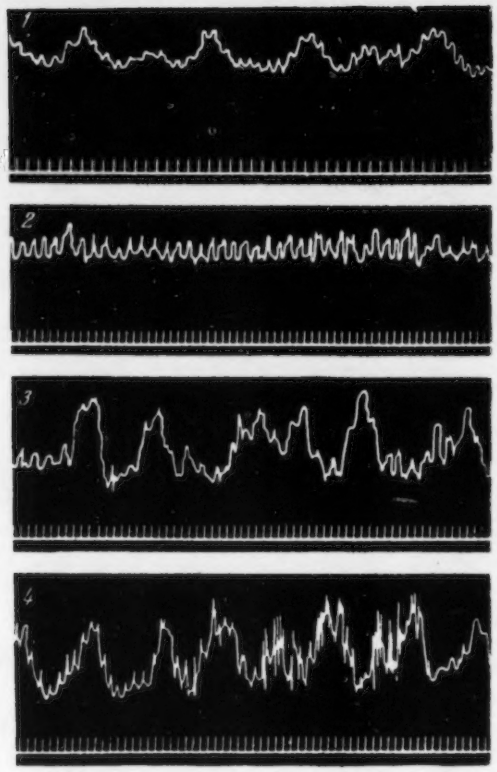

Fig. 3. Changes in gastric motor activity in experimental gastritis in the dog Dichka. All gastrograms recorded in the middle of the first hour of mechanical stimulation. 1) Normal motor activity, 2) 2nd day of gastritis, 3) 4th day of gastritis, 4) 20th day of gastritis. Time marker — 3 seconds.

SUMMARY

Experimental gastritis was induced in dogs by surface coagulation of the gastric mucosa with 10% silver nitrate solution. This gastritis was characterized by depression of the secretory function, inhibition of gastric peristalsis and increased tone of the stomach. The secretory function was re-established during the third month, the motor activity was restored at the end of the first month after coagulation of the gastric mucosa.

The secretory disturbances in experimental gastritis were found to be more considerable and lasting than derangement of gastric motility. This shows that the injury of the secretory glandular apparatus of the stomach was more pronounced.

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EFFECTS OF ADRENOCORTICOTROPHIC AND SOMATOTROPHIC HORMONES OF THE HYPOPHYSIS AND OF CORTISONE ON CERTAIN ASPECTS OF NITROGEN METABOLISM IN EXPERIMENTAL TOXIC HEPATITIS*

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It was found by one of us [3] that in disturbances of protein metabolism involving increased urinary output of nitrogen and intensification of hepatic proteolysis (experimental thyrotoxicosis, febrile states), the administration of adrenocorticotrophic hormone (ACTH), at certain dosage levels, and for certain periods of time, led to a fall in the urinary output of nitrogenous metabolites and in the intensity of the proteolytic process, i.e., it exerted an adaptive, so-called normalizing, effect [2]. For further development of this investigation we thought it necessary to ascertain whether ACTH exerts a similar effect in experimental toxic hepatitis, which is also associated with disturbances in the protein metabolism of the liver, expressed as enhanced breakdown of protein, with reduction of its synthesis. In view, also, of the known activating effect of somatotrophic hormone (STH) on protein synthesis [4], we thought it desirable to investigate its action under conditions of hepatic pathology.

The study of these questions is of some clinical importance, since it may provide data for the formulation of indications or counter-indications for the use of ACTH and somatotrophic hormone in patients suffering from liver disease.

EXPERIMENTAL METHOD

The experimental animals were white rats, weighing from 200 to 250 g. Experimental toxic hepatitis was induced by subcutaneous injection of 0.3 – 0.4 ml of CCl_4 per 100 g body weight (3 injections, given every other day). The animals were killed on the 3rd day after the last injection of CCl_4, i.e., on the 7th day from the 1st injection. Fatty degeneration of the liver was found in all the animals (fat content raised by 70-120% above normal). In the first series of experiments, daily subcutaneous injections of prolonged action ACTH (of Danish manufacture) were given together with the CCl_4, for 6 days, at dosage levels of 2 and 6 units. In the second series cortisone acetate (Roussel, Paris) was given instead of ACTH, in doses of 1 and 3 mg. In the third series of experiments we gave subcutaneous injections of somatotrophic hormone, prepared in the experimental manufacturing laboratory of our Institute, and assayed by the tibial growth test in the Department of Experimental Biology. The somatotrophic hormone was administered for 5 days, in daily doses of 1, 2, and 3 mg. The daily dose was divided into 2 parts, injected morning and evening.

We determined the protein content of the liver (from its nitrogen content), and we followed the incorporation of S-labelled methionine into the tissues and proteins of the liver of normal and CCl_4-poisoned animals, and of the latter during administration of ACTH and cortisone; the same was done for the liver, muscle, and kidney proteins of rats receiving STH. S^{35}-Methionine was injected intraperitoneally 24 hours before killing the animals, at dosage levels of 5000 – 6000 cpm per g body weight. Weighed portions of liver, muscle,

*Read at a meeting of the All-Union Institute of Experimental Endocrinology, March 19, 1957.

or kidney were homogenized, and were spread in a thick layer on aluminum sample pans, for activity measurements by means of an end-window counter. Preliminary experiments designed to construct the self-absorption curve for the tissues showed that in computing the results of measurements of thick samples the basis should be the activity of 12 mg of fresh tissue. Liver proteins were isolated by grinding the tissue with 10% trichloroacetic acid, filtering, washing the precipitate three times with the same solution, and then three times with methanol and ether. The product was then dried in a desiccator, and the activity of 50 mg portions was determined, in which case the thickness of the sample corresponded with 15 mg of protein, and this was taken into account in the calculations. The rate of incorporation of labelled atoms into the protein was expressed as percentages of radioactivity, in cpm in relation to cpm per g body weight, which was taken as 100%. Since the rate of incorporation of S^{35} into the proteins depended both on the rate of its supply to the liver cells and on the intensity of the process of its incorporation, we excluded the effect of the former factor (permeability changes) by calculating the relative activity of the protein from the following formula, proposed by D. E. Grodzenskii:

$$\frac{\text{activity of protein from 1 g of tissue (cpm)}}{\text{activity of 1 g of tissue (cpm)}}$$

The ratio provides a more correct assessment of the actual rate of incoporation of S^{35} into protein.

EXPERIMENTAL RESULTS

As is evident from the data presented in Table 1, administration of CCl_4, in doses of 0.3 ml per 100 g body weight given 3 times, every other day, leads to a fall in the protein content of the liver and in the rate of incorporation of methionine-S^{35} into its proteins. When 2 units daily of prolonged action ACTH were administered at the same time as the CCl_4 the protein content of the liver rose, as did the rate of incorporation of methionine-S^{35}. This effect was also seen with doses of 10 units of ACTH, although at this dosage level the protein content of the liver was not raised. Hence ACTH, at a certain dosage level (2 units), inhibits proteolytic processes proceeding in toxic hepatitis. At higher dosage levels (10 units), ACTH does not affect the protein content, although it still activates incorporation of methionine-S^{35} into hepatic proteins. It appears that large doses of ACTH stimulate both the processes of synthesis and of breakdown of protein, so that the resultant protein content is unchanged.

In the second group of experiments (Table 1, Group 6) we administered ACTH for 5 days, beginning with the day on which CCl_4 injection was discontinued, i.e., during the period of regeneration. The protein content of the liver of the control group (Group 5) remained low over this period, as did the relative activity of the protein, pointing to continuing inhibition of protein synthesis.* Under these experimental conditions, ACTH causes a rise in the relative activity of the protein, and slightly increases its content in the liver. In normal animals administration of 10 units of ACTH for 6 days causes a fall in the percentage incorporation of methionine-S^{35} into hepatic proteins, and in their relative activity.

Thus, at a given dosage level, ACTH retarded incorporation of methionine into the liver proteins of normal animals, but had the opposite effect in animals suffering from disturbances of assimilatory processes, as in toxic hepatitis. As in our previous work on thyrotoxicosis and febrile states, ACTH (at a certain dosage level) exerts an adaptive, "normalizing" effect in animals in which catabolic processes prevail in the protein metabolism of the liver, such as in CCl_4 poisoning.

When smaller doses of CCl_4 are administered (0.2 ml; 3 doses at 48 hr-intervals) the rate of incorporation of labelled methionine falls within the same limits as for normal animals, and in such animals the rate of incorporation into hepatic proteins is lowered by ACTH.

We next examined the effect of adrenal glucocorticoids, in order to ascertain to what extent the effect of ACTH on incorporation of methionine into liver proteins is due to stimulation of the adrenal cortex. We first administered cortisone to normal animals.

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*Increase in percentage incorporation of S^{35} into the proteins, with a fall in their relative activity, shows that the former effect is associated with intensification of entry of the labelled atoms into the tissue, but not with increase in the rate of their incorporation into the proteins.

TABLE 1

Effect of ACTH on the Protein Content of Liver and on Incorporation into the Proteins of Methionine-S^{35} in Normal Rats and in Experimental Toxic Hepatitis

Group	Nature of treatment	Protein content %	Percentage incorporation of S^{35} into proteins	Relative activity	No. of animals
1	Control, normal	21,0	8,95 (8,0—9,4)	0,54 (0,43—0,62)	12
2	CCl_4; 3 doses of 0.3 ml CCl_4 every other day	18,9	6,3 (5,3—6,7)	0,36 (0,29—0,4€)	14
3	CCl_4, 0.3 ml every second day + ACTH, 2 units daily	20,0	7,07 (4,3—9,4)	0,49 (0,40—0,60)	10
4	CCl_4, 0.3 ml every second day (3 doses) + ACTH, 10 units daily	18,8	7,74 (5,9—9,0)	0,47 (0,29—0,60)	14
5	CCl_4, 0.3 ml, as before (3 doses); rats killed 5 days after the last injection	16,9	8,3 (7,3—9,9)	0,35 (0,34—0,38)	7
6	The same, + ACTH, 10 units for 5 days after the last injection of CCl_4	17,3	8,1 (6,9—7,7)	0,44 (0,36—0,52)	8
7	ACTH, 10 units daily for 6 days	21,2	7,65 (7,1—8,2)	0,39 (0,37—0,42)	6
8	CCl_4, 0.2 ml, 3 doses, given (every second day)	21,0	7,3 (5,3—9,1)	0,55 (0,52—0,58)	9
9	CCl_4, 3 doses of 0.2 ml, given every second day + ACTH, 10 units daily	20,5	5,6 (4,6—6,6)	0,46 (0,43—0,48)	9

Note: Mean values, range shown in parentheses.

It appears from the data of Table 2 that administration of cortisone to normal rats, at a rate of 1 mg daily for 6 days, leads in most cases to lowering of the protein content of the liver, and of its relative activity, while percentage inclusion of labelled methionine remains at the normal level. These effects can be interpreted as giving evidence of an increase in the rate of entry of methionine into the hepatic tissues. As has been shown above, the actual rate of incorporation of labelled methionine into liver proteins is given by the relative activity. Daily injection of 3 mg of cortisone acetate for 6 days lowers both the relative activity of the liver proteins and the percentage incorporation of labelled methionine into the proteins.

M. B. Lebedeva [1] administered cortisone in dosages of 5-7.5 mg for 2 days, and found that the rate of incorporation of glycine-1-C^{14} into liver proteins was raised for 1-6 hours after the injections. When cortisone was given in daily doses of 2.5 mg for 6 days, and of 5 mg on the 7th day, and the animals were killed 6 hours after administration of glycine, she found that the rate of incorporation of glycine into liver proteins was lowered, as was the total nitrogen content of the liver; these results are similar to those found by us in the study of the effect of injecting cortisone acetate, at dosage levels of 3 mg per diem daily for 6 days, on the rate of incorporation of methionine-S^{35} into liver proteins, 24 hours after its intraperitoneal injection.

Injection of cortisone (1 mg daily for 6 days) simultaneously with injection of CCl_4 raised the rate of incorporation of methionine-S^{35} into liver proteins (Table 2), i.e., it had the same effect as ACTH. There was, however, no increase in the protein content of the liver, showing that protein anabolic processes were balanced by catabolic ones. At higher dosage rates of cortisone (3 mg daily) we found a fall in the protein content of liver, and of percentage incorporation into the proteins of methionine; the relative activity did not rise. It thus appears that relatively high doses of cortisone (in contrast to small ones) did not stimulate protein anabolic processes in toxic hepatitis.

Injection of somatotrophic hormone at the same time as CCl_4, in doses of 1, 2, and 3 mg daily for 5 days (Table 3), prevents the halt in increase of body weight found in the control animals. The protein content of the liver rose slightly at STH dosages of 1 and 2 mg, but did not change at dosage levels of 3 mg. Percentage incorporation of methionine-S^{35} into liver proteins, and their relative activity, rose slightly after administration of STH at a dosage level of 2 mg*, but showed no change at dosage levels of 1 and 3 mg. We could find no changes in the protein content of muscle, percentage incorporation therein of methionine, or in the relative activity of the proteins, after administration of STH. The protein content of kidney tissue, the percentage incorporation therein of labelled methionine, and the relative activity of the proteins rose at dosage levels of 1 mg of STH; at a dosage level of 2 mg the protein content rose somewhat, while percentage incorporation of methionine and the relative activity of the proteins remained unchanged. At a dosage level of 3 mg of STH both the protein content of the kidneys, and the relative activity of the proteins fell.

TABLE 2

Effect of Cortisone on the Protein Content of Liver, and on the Incorporation into Liver Proteins of Methionine-S^{35} in Normal Rats and in Experimental Toxic Hepatitis

Group	Nature of treatment	No. of animals	Body weight (g)		Protein content (%)	Percentage incorporation of S^{35} into proteins	Relative activity
			initial	final			
1	Control, normal	12	—	—	21,0	8,95 (8,0—9,4)	0,54 (0,43—0,62)
2	Cortisone, 1 mg daily for 6 days	10	234	237	19,0	8,70 (6,5—10,8)	0,44 (0,34—0,54)
3	Cortisone, 3 mg daily for 6 days	12	221	215	20,0	6,5 (5,0—7,7)	0,47 (0,34—0,54)
4	CCl_4, 0.3 ml, 3 doses given every other day	14	225	214	18,9	6,3 (5,3—6,7)	0,36 (0,29—0,46)
5	The same, + cortisone, 1 mg daily for 6 days	8	237	224	19,0	8,2 (6,3—10,4)	0,44 (0,40—0,49)
6	CCl_4, 0.3 ml (3 doses, given every other day) + cortisone, 3 mg daily for 6 days	12	220	202	17,8	4,6 (3,1—6,4)	0,34 (0,28—0,41)

Note: Mean values; range shown in parentheses.

It thus appears that in experimental toxic hepatitis the effects produced by our STH preparation on protein anabolism vary, according to the dose given and to the organ examined. At a dosage level of 2 mg STH exerts an activating effect on protein synthesis in the liver; this effect is seen with doses of 1 mg in the kidneys. A dosage level of 3 mg even lowers the protein content of the kidney. This effect might possibly be due to the presence of thyrotrophic hormone in our preparation, which could have an appreciable action at the higher dosage levels taken by us. The STH preparations used by us had no effect on protein synthesis in muscle. It should be noted that in those cases in which STH administration had no effect on the percentage content of protein in the liver, muscles, and kidneys, its absolute amount was increased, owing to increase in weight of the organs and tissues under examination.

*At this dosage level of STH we have observed some activation of protein synthesis in the liver of normal animals.

TABLE 3

Effect of Pituitary Somatotrophic Hormone (STH) on the Protein Content of the Liver, Muscles, and Kidneys, and on Incorporation of Methionine-S^{35} into the Proteins (mean values)

Nature of treatment	No. of animals	Body weight (g)			Liver			Muscles			Kidneys		
		initial	final	difference	proteins (%)	% incorporation of S^{35} into proteins	relative activity	proteins (%)	% incorporation of S^{35} into proteins	relative activity	proteins (%)	% incorporation of S^{35} into proteins	relative activity
CCl_4, 3 doses of 0.3 ml, every other day	14	219	215	—4	17,9	6,42	0,31	19,3	1,80	0,25	15,9	12,0	0,32
CCl_4, 3 doses of 0.3 ml every other day + STH, 1 mg daily for 5 days	14	216	228	+12	19,4	6,35	0,32	20,0	1,73	0,26	16,8	16,0	0,44
CCl_4, 3 doses of 0.4 ml every other day	13	210	198	—12	18,9	6,13	0,26 $m \pm 0,015$	20,2	1,74	0,30	16,7	13,1	0,35
CCl_4, 3 doses of 0.4 ml every other day + STH, 2 mg daily for 5 days	13	212	216	+4	19,9	7,0	0,33 $m \pm 0,016$ $t=3,7$	20,7	1,78	0,30	17,8	13,8	0,36
CCl_4, 3 doses of 0.4 ml every other day	8	205	203	—2	17,4	4,91	0,24	18,0	1,47	0,20	15,7	12,3	0,32
CCl_4, 3 doses of 0.4 ml every other day + STH, 2 mg daily for 5 days	7	205	213	+8	17,7	5,10	0,26	18,7	1,40	0,24	13,9	12,2	0,26

SUMMARY

In experimental toxic hepatitis of rats caused by administration of CCl_4 a fall is found in the protein content of the liver, and in the rate of incorporation of methionine-S^{35} into these proteins. When prolonged action ACTH (2-10 minutes) or cortisone (1 mg) are injected at the same time as the CCl_4 the rate of incorporation of methionine-S^{35} is raised. Higher doses (3 mg) of cortisone had the opposite effect. Administration of somatotrophic hormone prevents loss of weight of CCl_4-poisoned rats. Only a small rise in the protein content of the liver, and in the rate of incorporation of methionine-S^{35} into the proteins were found at a dosage level of 2 mg of STH. The protein content of the kidneys, and the rate of incorporation of methionine-S^{35} are increased with dosage levels of 1 mg of STH. At a dosage level of 3 mg a fall in the protein content of the kidneys was found. Administration of STH did not affect the protein content of muscles, or the rate of incorporation of methionine-S^{35} into the muscle proteins.

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THE FUNCTIONAL STATE OF THE NERVE–MUSCLE PREPARATION IN THE INTACT ORGANISM UNDER CONDITIONS OF DISTURBED CIRCULATION

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Investigation of the natural course of development of muscle ischemia due to disturbance in circulation is of considerable interest both practically and theoretically. Of parameters typifying the functional state of the nerve – muscle apparatus under conditions of ischemia those studied in greatest detail are rheobase and chronaxy [1, 2, 5, 7, 8, 9, 10, 11, 12] and also accommodation [9]. However, such important indices as refractivity and lability were not subjected to investigation. Estimation of lability based only on the threshold reaction to rhythmic stimulation [11] or on the degree of chronaxy can hardly be regarded as convincing. Chronaxy, threshold reaction [6] and even refractivity determined with the aid of two consecutive impulses cannot serve as criteria regarding capacity of nerve – muscle preparations for rhythmic stimulation and tetanic contraction. By applying the method developed in the laboratory for incomplete isolation of muscle [3], we studied the lability of the nerve – muscle preparation and its capacity for summation of two consecutive impulses upon abrupt disturbance of circulation within the muscle.

EXPERIMENTAL METHOD

Lability was determined with the aid of rhythmic stimulation with square-wave impulses of 0.2-millisecond duration and a frequency rising from 10 to 2500 impulses per second, suitability for summation – with the aid of two maximal impulses of the same duration. The interval between them was gradually increased to the duration of 3 diapasons.* During each diapason a series of tests was carried out. Interval between impulses in the first diapason for the first test was less than 0.1 millisecond, for the subsequent ones – 0.2; 0.3; 0.4; 0.6; 0.8; 1; 1.2; 1.4; 1.6 and 1.7 milliseconds; in the second diapason – for the first test – 1 millisecond, for subsequent ones – 1.5; 2; 3; 4; 5; 6; 7; 9 and 11 milliseconds, and in some instances up to 15, 18, 22 and 25 milliseconds; in the third diapason – 5, 7, 12, 17, 22, 30, 40, 50, 60, 70, 85, 100 and 110 milliseconds; in some instances 7 and 17 millisecond intervals were eliminated in which cases the third diapason consisted of 11 tests. As the interval was increased the phase of absence of summation of stimulation made its appearance (phase of absolute refractivity) and lasted 0.6-0.8 milliseconds; also the phase of increasing summation (intervals from 0.6-0.8 to 1.5-2 milliseconds), the phase of maximal summation (intervals from 1.5-2 to 18-20 milliseconds), phase of decreasing summation (intervals above 20 milliseconds) (Fig. 1). Unipolar stimulation was used in the experiments. Active platinum electrodes (epicutaneous and needle-shaped) were placed upon or into a muff which contained a motor nerve. For direct stimulation the electrodes were inserted hypodermically at the cranial and caudal ends of the muscle contained in the muff. Muscle contraction was recorded on the kymograph. Ischemia was produced by clamping (for up to 3 hours) the vascular muffs and both ends of the muscle (at the points of its attachments) with small clamps. In the course of examination of muscular contraction, this technic makes it possible to eliminate undesirable moments which are unavoidable with other technics (anesthesia and trauma in acute experiments; compression of a nerve with a tourniquet; incomplete ischemia from the use of straps, etc.). The parameters were recorded during the experiment every 2-3 minutes.

*As in original.

The total number of experiments was 23. Lability was investigated 18 times, summation capacity – 13.

EXPERIMENTAL RESULTS

The contractile effect of muscle in response to rhythmic and single impulses decreased progressively as the degree of ischemia progressed. We did not observe phases of increased excitability and contractile capacity of muscle, as described by some workers [7, 11] and not elicited by others [9, 10]. Indirect excitability of the nerve – muscle preparation disappeared 20-30 minutes following application of clamps to the vessels (first stage ischemia). Sensitivity of muscle to direct stimulation, although gradually decreasing, was preserved for $1\frac{1}{2}$ – 2 hours (second stage ischemia).

A progressing increase in the phase of absolute refractivity was observed in all experiments 3-5 minutes after clamping of the vessels. Prior to the disappearance of the neuromuscular conductivity this parameter increased from 0.6-0.8 milliseconds to $1\frac{1}{2}$ – 2 milliseconds, i.e., twice or more. The phase of increasing summation shifted in the direction of longer intervals and increased by 1 millisecond. The interval at which beginning of decreasing summation occurred did not change. The phase of maximal summation was thus shortened by $1\frac{1}{2}$ – 2 milliseconds. These phase changes took place during the first 15-20 minutes. As ischemia deepened there developed a disturbance in summation of impulses which followed one another at optimum intervals (3-16 milliseconds). Not long before the disappearance of neuromuscular conductivity the responses of the muscle to two impulses equalized regardless of the intervals between the latter, i.e., there developed a loss of capacity for summation of two indirect stimulations (see Fig. 1, e).

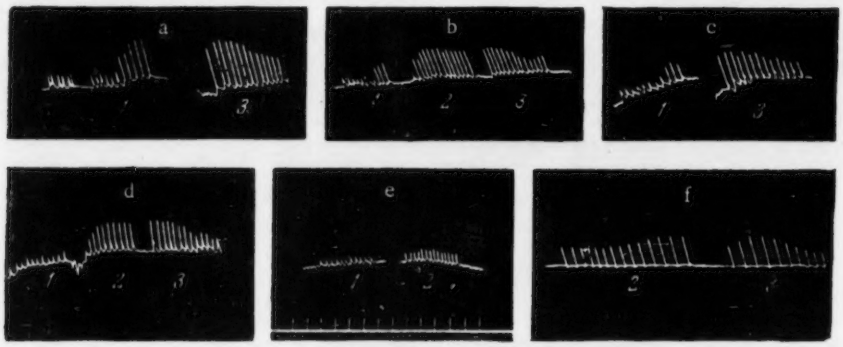

Fig. 1. Changes in capacity for summation in the nerve – muscle preparation in a state of ischemia. a) Initially, b) seven minutes after application of clamps, c) ten minutes later, d) eighteen minutes later, e) twenty minutes later, f) thirty-seven minutes later. Time – five seconds. The numbers below the contraction tracings identify the group of tests (diapason). Contractions on kymogram f were recorded by direct stimulation of muscle.

In the second stage of ischemia, during which sensitivity of the nerve – muscle preparation was preserved only to direct stimulation, increase in the refractory phase continued as brought forth now with the aid of direct stimulation. It reached 6-10 milliseconds and over. The maximal summation phase was shortened to 2-4 instead of 17-20 milliseconds, and was displaced in the direction of intervals of 20 milliseconds and over (see Fig. 1, f).

During the entire first stage of ischemia and up to the point of disappearance of indirect excitability, the lability of the nerve – muscle preparation, as far as our technic permits us to judge, did not experience any substantial changes. Optimum of muscle contraction developed at the same frequency of stimulation (Fig. 2). Only in the second stage, when even direct excitability decreased to the point that in order to obtain muscle contraction stimuli of 20-40 v were required, there developed a decrease in lability which gradually progressed. Optimum muscle contraction arose at 30-50 impulses per second instead of 70-100 (Figs. 2 and 3). Frequency gradually decreased followed by normal contraction. Thus, at first tetanic contraction followed in

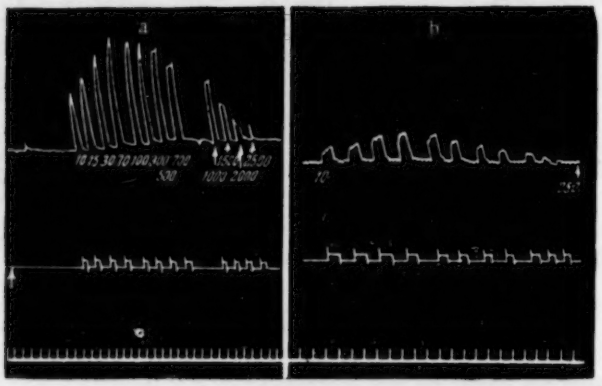

Fig. 2. Lability of a nerve – muscle preparation in the first stage of ischemia (indirect stimulation). Meaning of tracings (from above down): muscle contractions, record of stimulation, record of time (5 seconds). a) Initial lability, b) 20 minutes after cessation of circulation. The numbers beneath the contraction tracings indicate the frequency of stimulation in terms of impulses per second.

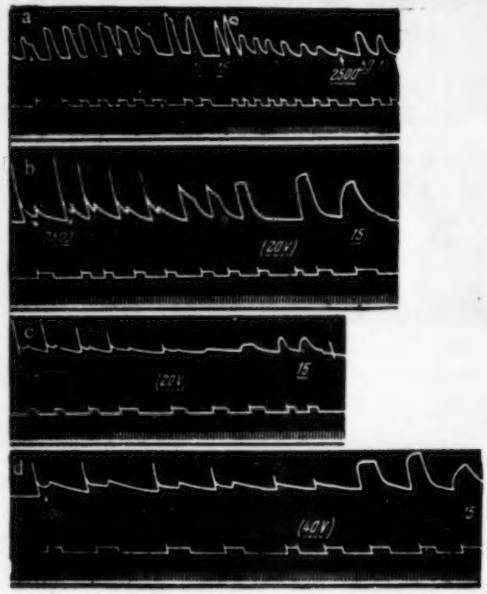

Fig. 3. Changes in lability of a nerve – muscle preparation in the second stage of ischemia (direct stimulation). a) Lability 45 minutes after onset of ischemia, b) 65 minutes later, c and d) 1 hour and 45 minutes later. Frequency of stimulation (the numbers beneath the contraction tracings) 15, 50, 100, 300, 500, 1000, 1500, 2000, 2500 impulses per second. Numbers in parentheses indicate the amplitude of stimulation in volts.

response to rhythmic stimulation at a rate as high as 1,500 impulses per second, later in response to stimulation of not more than 500 impulses per second and finally, in response to stimulation of not more than 100

impulses per second (Fig. 3). To stimulation of greater frequency the muscle reacted with an initial twitching which changed in succession first to a decreasing tonus then an increasing one when the current was disconnected and finally to a tonic contraction. Such a reaction reminds one of the response of normal muscle to the passage of a continuous current. Apparently, the ischemic muscle loses the capacity of rhythmic excitability and responds to stimulation of even moderate frqquency such as stimulation with a continuous current. It is interesting to note that the greater the frequency of stimulation the greater the contractile response of the muscle in the form of the initial twitching, i.e., the degree of contraction is determined by the quantity of electricity passing through an organ per unit time, which increases together with a rise in the frequency of the impulse. Simultaneously, other changes in the contractile reaction of muscle take place, such as slowing down of contraction and especially its weakening after a tetanic contraction or an isolated twitching (residual contracture).

In such a manner, the disappearance of neuromuscular conductivity in ischemia of the skeletal muscle of the rabbit is accompanied by a decrease in excitability of the nerve – muscle preparation and a disturbance of summation of two stimulations. Abrupt changes in lability which could be uncovered by our technic were not observed by us. After cessation of nerve – muscle conductivity, the disturbance in excitability and capacity for summation continues to deepen and in addition there is an abrupt fall in lability. A reactivity of muscle develops in $1\frac{1}{2} - 2$ hours after complete ischemia. Changes in lability, capacity for summation, excitability and contractile capacity of a muscle in a state of ischemia differ from changes in these parameters in other forms of disturbance of the nerve – muscle preparation due, for example, to curare-like substances or anesthesia [4].

SUMMARY

Experiments were staged on rabbits with the aid of a method of incomplete separation of the muscle and placing it into a cutaneous muff with intact vascular and nervous connections. The lability and contractile property of the nerve – muscle preparation, preserved in the body, was studied in the state of ischemia. The decrease of excitability, increase of the absolute refractory phase and shortening of the phase of maximal summation were noted under the effect of ischemia. These changes progressed after development of the block of the neuromuscular transmission and an acute decrease of lability was added. Complex reactivity of the muscle appeared in $1 - 1\frac{1}{2}$ to 2 hours of ischemia.

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BIOCHEMISTRY AND BIOPHYSICS

THE NUCLEIC ACIDS, PHOSPHOLIPIDS AND PHOSPHOPROTEINS OF THE HUMAN LEUCOCYTE

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The biochemical study of leucocytes is continuously expanding to include almost all aspects of the metabolism of these cells, which are of utmost importance in the maintenance of the normal state of the animal body. Nevertheless, the data presently available on the white blood cells are both incomplete and contradictory. This is particularly true for the acid-insoluble phosphorus compounds of the leucocytes, namely, the nucleic acids, phospholipids and phosphoproteins. Certain data, even though contradictory, dealing with the quantitative content of nucleic acids and phosphoproteins in the leucocytes, have recently been published [3, 4, 6] but there is practically no information on the turnover rates of these compounds.

It was shown in a previous communication from this laboratory [1] that a powerful glycolytic mechanism for the splitting of sugar existed in the human leucocyte, and that this mechanism could completely satisfy the cell's energetic requirement under anaerobic conditions. The extent of glycolysis in the granulocytes is so large, that it is not completely suppressed in the presence of atmospheric oxygen, as is the case with other cells, and subsequently, under normal physiological conditions, respiration and glycolysis coexist. Furthermore, respiration was shown to be partially suppressed in the presence of intensified glycolysis, with the result that oxygen uptake was lower in the presence of glucose than in its absence (so-called reverse Pasteur effect) [1]. These peculiarities in the metabolism of leucocytes set them metabolically apart from other cells of the animal body, and are reminiscent of the metabolic patterns of malignant cells.

However, the observations listed above dealt but with the very basis of the energy metabolism – respiration, glycolysis and adenosinetriphosphate (ATP) turnover. It was considered of importance to gain further insight into the biochemical characteristics of the human white blood cells, and primarily of the facets of metabolism concerned with their growth and function. Since the cellular synthetic activity, growth, division, as well as some other functions, are connected with the turnover of nucleic acids and, possibly, of other acid-insoluble organophosphates, we have undertaken a study of the quantitative content and the turnover rates of the phosphorus moiety of the ribonucleic acids (RNA), desoxyribonucleic acids (DNA), phospholipids (PL) and phosphoproteins (PP) in the various types of human leucocytes, in normal subjects and in certain pathological conditions. Corresponding investigations were carried out with highly purified leucocyte suspensions from normal human subjects, and from patients suffering from both chronic and acute myeloid leukemia, and from chronic lymphadenosis.

EXPERIMENTAL METHODS

Leucocyte suspensions in human serum containing no more than 10% red blood cells were incubated with shaking in the presence of glucose and radioactive phosphate (360,000 cpm per ml of incubate) in Warburg vessels at 37°, under aerobic and anaerobic conditions. At the end of the incubation period the cells were separated and washed, and analyzed for inorganic phosphate, ATP, RNA, DNA, PL and PP. The nucleotides

The Quantitative Content of the Phosphorus of Various Compounds in the Human Leucocyte and Their Turnover Rates

	Phosphorus													
	ATP		RNA			DNA	DNA		PL			PP		
				RSA			RSA			RSA			RSA	
	γ	RSA	γ	I	II	γ	I	II	γ	I	II	γ	I	II
1. Normal donor														
Aerobic	636±22	63±5	1191±51	3,3±0,7	5,9±0,2	4235±278	0,17±0,03	0,25±0,05	2 60±135	2,4±0,4	2,7±0,5	96±14	10±2,6	13∓3,1
Anaerobic	636±26	62±5	1165±134	4,8±0,5	6,4±1,0	3960±283	0,20±0,03	0,31±0,04	2490±235	1,7±0,3	1,9±0,3	102±20	12±1,7	18±3,0
1. Chronic myeloid leukemia														
Aerobic	621±36	71±3,5	1950±179	3,0±0,6	3,9±0,05	4351±299	0,17±0,02	0,23±0,02	2570±230	0,8±0,3	1,3±0,2	111±30	10,1±1,9	14,9±2,5
Anaerobic	644±38	70±4,6	1780±193	3,8±0,6	4,7±0,6	4900±336	0,14±0,01	0,17±0,02	2360±189	1,2±0,2	1,2±0,3	106±24	11,5±1,3	15,6±1,2
2. Acute myeloid														
Aerobic	740±66	65±5,6	3590±453	1,8±0,2	2,6±0,4	6750±440	0,13±0,02	0,15±0,02	—	—	—	167±45	14±2,6	18±2,4
Anaerobic	760±72	64±5,2	3150±420	2,1±0,4	2,6±0,4	6580±600	0,11±0,02	0,14±0,02	—	—	—	187±25	11±1,5	14±1,6
3. Lymphadenosis														
Aerobic	736±40	83 5,4	4043±210	2,3±0,3	3,0±0,11	12940±595	0,25±0,08	0,31±0,06	2943±318	1,0±0,06	2,1±0,8	143±24	13,5±1,7	18±2,4
Anaerobic	780±47	83 - 9,2	3530±100	2,4±0,2	3,0±0,4	12980±920	0,34±0,1	0,39±0,1	2875±319	1,4±0,07	1,5±0,6	131±17	15,8±1,9	22,4±3,7

Values expressed as γ phosphorus per 1 g of dry cell weight. Incubation time, 60 min. temperature 37°; cell suspensions in serum.

$$\text{RSA I} = \frac{\text{Specific activity of the fraction phosphorus}}{\text{Specific activity of the inorganic phosphorus}}; \quad \text{RSA II} = \frac{\text{Specific activity of the fraction phosphorus}}{\text{Specific activity of the ATP-phosphorus}}.$$

[2] Incubation time — 30 min.

(ATP) were adsorbed onto charcoal [2], the acid-insoluble compounds were fractionated according to Schmidt-Thannhauser [5]. The specific activity (SA) of the phosphorus of all the fractions mentioned was determined, and the relative specific activity (RSA) of the fractions was calculated as follows: I – with respect to intracellular inorganic phosphorus (RSA-I); II – with respect to the phosphorus of the immediate precursor of most of the compounds investigated – ATP (RSA-II).

EXPERIMENTAL RESULTS

Presented in the Table are the results of the quantitative assays and turnover rate determinations on the phosphorus of ATP, RNA, DNA, PL and PP in various groups of human leucocytes, incubated for one hour at 37°, in human serum under aerobic and anaerobic conditions (as an exception from general procedure, the leucocytes from patients suffering from acute myeloid leukemia were incubated for 30 min in view of their elevated metabolic activity).

It is noteworthy, with respect to the content of the various organophosphates in the leucocytes, that there was little deviation in the PP and PL content of leucocytes in the various groups investigated. On the other hand, the RNA phosphorus content varied widely, its lymphocyte content being twice that of leucocytes of chronic myeloid leukemia patients, and nearly four times that of normal human leucocytes. The DNA-phosphorus content of normal leucocytes was approximately the same as that of leuocyctes of chronic myeloid leukemia patients. The DNA-phosphorus content of lymphocytes was found to be very high, being about three times that of the normal leucocytes. This is quite understandable in view of the mass ratio of the nucleus and the whole cell in lymphocytes. There was a considerable increase in the RNA-phosphorus content, and some increase in the DNA-phosphorus content (compared with the normal leucocytes) in the acute leukemia group. No significant differences between the ATP contents of the various cell groups were found.

It is obvious from the results of the tracer experiments that the least metabolically active fraction was that of DNA; the RSA value of its phosphorus was less than one per cent of the intracellular inorganic phosphorus, and only slightly higher when calculated on the basis of ATP-phosphorus. The rate of phosphorus rejuvenation in the PL fraction was found to be considerably higher, the RSA value being 1-2.4%. The RNA phosphorus was found to exchange at still higher rates, the RSA values obtained in the various leucocyte groups, ranging from 1.8 to 4.8%. The rate of the PP-phosphorus exchange was found to be exceptionally high. The RSA values of this fraction reached 10-15%.

Of all the leucocyte groups investigated, the normal leucocytes showed the highest degree of phosphorus turnover in the RNA and PL fractions, while the cells obtained from lymphadenosis patients showed the highest turnover of phosphorus in the DNA and PP fractions. The rate of ATP-phosphorus turnover was highest in the leucocytes taken from acute leukemia patients, and slightly lower in the lymphocytes.

It was mentioned above that the metabolism of all the human leucocyte types under anaerobic conditions was energetically equivalent to their metabolism in the presence of air. This has now found confirmation in the present study of the behavior of the complex intracellular phosphorus-containing compounds of the leucocyte – nucleic acids, PL and PP. As will be seen from the Table, the rate of phosphorus turnover in these compounds was found to be practically the same under aerobic and anaerobic conditions. The glycolytic cleavage of sugar in the absence of air was apparently capable to support energetically the complete resynthesis of even the most complex high molecular weight compounds of the human white blood cells.

The effective ATP resynthesis, and the satisfactory turnover of the nucleic acid-, PL- and PP-phosphorus in the leucocytes under anaerobic conditions demonstrated the excellent adaptability of these cells to possible oxygen deficiency, and their ability to carry out completely the necessary specific functions under conditions of deficiency, or complete absence, of atmospheric oxygen.

SUMMARY

Leucocytes in a healthy man contain the following (measured in gamma of phosphorus per 1 g dry weight of the cells): ribonucleic acid – 1191 ± 51; desoxyribonucleic acid – 4235 ± 278; phospholipids – 2760 ± ± 135; phosphoproteins – 96 ± 14. Leucocytes from the blood of patients with chronic myeloid leukemia contain correspondingly with the above: 1950 ± 179; 435 ± 299; 257 ± 230 and 111 ± 30. The quantity of phosphorus in the same compounds of leucocytes in the patients with chronic lymphadenosis equals 4043 ± 210;

12,940 ± 595; 2943 ± 318 and 143 ± 24, respectively. In acute leukemia: RNA – 3590 ± 453; DNA – 6750 ± 440; phosphoproteins – 167 ± 45. Phosphorus exchange is most intense in phosphoproteins and the least so in DNA, while RNA and phospholipids occupy a middle position. The rapidity of phosphorus exchange in all of these compounds does not depend on the conditions of aeration. Resynthesis of adenosinetriphosphate and phosphorus exchange in nucleic acids, phospholipids, and of phosphoproteins in leucocytes is carried out in anaerobic conditions in the same way as in aerobic.

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RIBONUCLEIC ACID IN PERIPHERAL BLOOD CELLS

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The present work deals with the ribonucleic acid (RNA) content of the peripheral blood cells of the normal horse.

Published work on the RNA content of the blood cells and of the hematopoietic tissue cells has established that large amounts of RNA were present in the lymphoblasts and myeloblasts [1], myeloblasts and erythroblasts [5] and in hemocytoblasts [7].

During the "maturing" of hemoglobin in the erythroblastic elements, and of the specific granulation in the myeloid elements, the RNA content of these cells was observed to decrease [2]. Some workers claim that RNA is also present in the lymphocytes of rats [5] guinea pigs [6] monkeys [8] and in human lymphocytes [4]. According to E. I. Terent'eva, human lymphocytes contain little or no RNA. However, a search of the literature failed to reveal any published data on the RNA content of the peripheral blood cells of the horse.

EXPERIMENTAL METHODS

Two blood samples were taken from each of healthy, nonimmunized horses. The RNA content of the cells was determined according to Brachet (using ribonuclease and hot water for the controls). Usual clinical blood analysis was also carried out.

EXPERIMENTAL RESULTS

The RNA was found in the cytoplasm of monocytes and lymphocytes, where it forms fine, irregular granules. Some of the granules stained a deep red with pyronin , while others stained only faintly, which may be due to the different RNA content within the various granules. The monocyte cytoplasm was found to contain the largest amount of RNA granules; their content was sometimes sufficiently high to obscure the chromatin of the nucleus.

In the lymphocyte the RNA granules were distributed mainly on the periphery of the cytoplasm. Odd granules were frequently found on the cell surface. On the basis of the individual cell's granule content the lymphocytes may be divided into three groups. The first group was characterized by a very high content of RNA granules. Their entire (or nearly entire) cytoplasm was densely filled with granules; the granules thickly surrounded the nucleus. The second group comprised lymphocytes in which the granules occupied a more or less significant portion of the cytoplasm. Here the granules were found mainly on the cytoplasmic periphery, leaving the perinuclear portion free, while only few granules could be found on the nuclear surface. The third group comprised lymphocytes containing only few single granules (see Figure). No RNA in granular form could be found in other hematopoietic cells. Presented in the Table are the statistical data of the mean per cent distribution, and the absolute number of lymphocytes containing large, moderate, and small amounts of granulated RNA, together with the standard deviation. The separation of lymphocytes into groups was carried out in each count of 100 cells in smears.

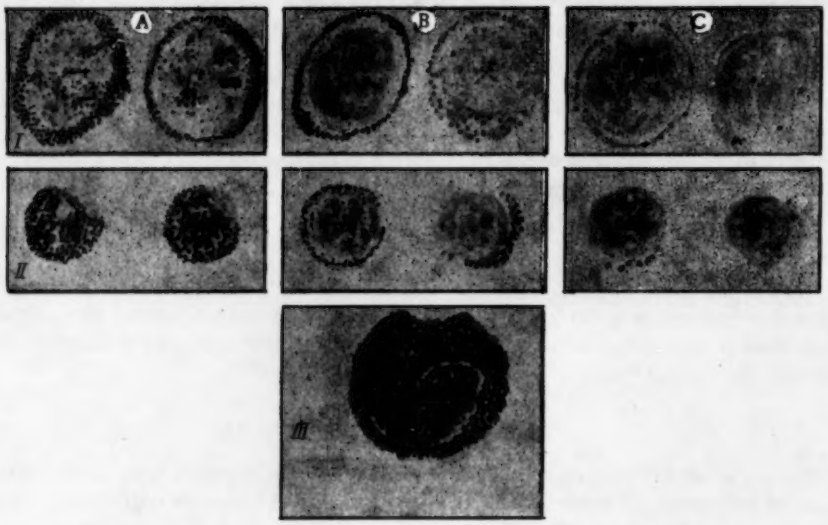
The observed differences in the degree of saturation of the lymphocytic cytoplasm with RNA, irrespective of cell size, shows that there exist cells, among both the large and small lymphocytes, with varying potential capacities for the carrying out of processes whose intensity depends on the cell's RNA content.

The Mean Per Cent Distribution and Absolute Numbers of Lymphocytes with Various Granulated RNA Content

Mean lymphocyte count per ml blood	Per cent lymphocytes containing RNA in			Numbers of lymphocytes per ml blood, containing RNA in		
	large amounts	moderate amounts	low amounts	large amounts	moderate amounts	low amounts
3234±912	11±2.2	85±2.7	4±2	363±105	2748±87	125±59

The possible function of RNA in the lymphocytes and monocytes requires further study. However, as found in other experiments in this laboratory, the RNA content of blood cells increased during immunogenesis. These results will be presented in more detail in a further communication.

The present work showed that the monocytes of horse blood contain large quantities of RNA granules.



The demonstration of ribonucleic acid (RNA) in horse lymphocytes and monocytes. I) Large lymphocytes, II) small lymphocytes, III) monocyte. A) Large quantity of RNA granules, B) moderate quantity of RNA granules, C) small quantity of RNA granules.

The RNA granule content of the lymphocytes was found to vary with individual cells, ranging from very high to but a few granules in some cells.

The difference in degree of saturation with RNA in the lymphocytic cytoplasm shows that individual lymphocytes vary in their activity with respect to RNA-dependent functions. No RNA-containing granules were found in other blood cells.

SUMMARY

The presence of RNA was investigated in the cells of peripheral blood of horses by Brachet's method. Monocytes contain many RNA granules, while the quantity of RNA granules in lymphocytes varies from single granules to a large number of them. Thus, it was demonstrated by the histochemical method that lymphocytes

possess different activity with regard to the functions caused by RNA. No RNA granules were revealed in other blood cells. The authors estimated the number of lymphocytes rich in RNA, of those containing a moderate amount, and those with only a few RNA granules.

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ON THE BIOENERGETICS OF BILE SECRETION

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According to recent communications [6-8] on the mechanism of bile formation, it appears that this process consists of the active secretion of the elements of bile by the liver cells, followed by filtration from the blood stream via capillary membranes and a slight resorption of water and some other bile ingredients form the bile ducts. Bile formation through secretion is obviously an energetic process. These considerations prompted the present study of the bioenergetics of bile formation and of the action of some cholagogues, widely applied in the treatment of liver disease with demonstrable dysfunction of bile secretion.

The present communication deals with some problems of the chemical dynamics of bile formation.

EXPERIMENTAL METHODS

Isolated cat liver preparations were made according to accepted procedure. The afferent cannula was placed into the portal vein; the bile was collected through a cannula placed in the main bile duct. The liver was perfused with physiological saline, previously aerated and warmed to 39°. Perfusion was carried out under a hydrostatic pressure of a 20 cm column of water. The rate of bile secretion was determined over a two hour period, and was expressed in ml per 30 min. Inhibitors were employed at the following concentrations: Sodium cyanide – M/500, monoiodoacetic acid – M/2000, sodium fluoride – M/100, 2,4-dinitrophenol – M/700 and sodium arsenate – M/100.

In situ experiments were carried out either with rabbits or with dogs prepared with either a gall-bladder fistula or a main bile duct fistula (in the latter case the bladder duct was ligated). Sodium fluoride was given intravenously in doses 30 mg/kg.

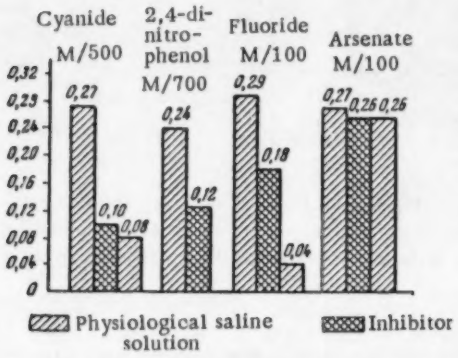
In experiments dealing with liver oxidation and phosphorylation, aliquots of rabbit liver homogenate (400 mg) were incubated for 40 min at 26° in Warburg vessels containing phosphate-salt mixture (pH 8.1-8.2), 15 mg of glucose, and 6.5 mg of sodium fluoride in a total volume of 3 ml. Incubations were carried out under both aerobic (oxygen) and anaerobic (hydrogen) conditions. Intensity of phosphorylation was determined by the uptake of inorganic phosphate, the remainder of which was estimated in the trichloroacetic acid extract of the incubation mixture using the molybdate reagent and eikonogen.

Adenosinetriphosphate and inorganic orthophosphate were estimated in liver tissue frozen in liquid air. Inorganic phosphate (P_0) in the trichloroacetic acid extract was determined after precipitation with magnesia mixture, and the labile phosphate of ATP etc. – as the difference between P_7 mineral phosphate after 7 min at 100° in 1 N HCl) and P_0. Using a specific enzymatic method and the preparative isolation of ATP from liver, G. P. Toropova [5] showed that the "pyrophosphate fraction" of liver, as determined by acid hydrolysis, represented the true ATP content of this tissue. The high glycogen content of liver did not interfere with ATP determinations.

EXPERIMENTAL RESULTS

Isolated livers were perfused with physiological saline for two hours in each of six independent experiments. The rate of bile secretion was found to be fairly stable throughout; only in one case there occurred a spontaneous decrease of secretion rate toward the end of the second hour.

Whenever inhibitors were employed, the normal secretion rate was first determined over a 30-min period, and the inhibitor was then introduced with the perfusate. Tissue respiration was depressed by sodium cyanide. The completeness of the respiratory block was confirmed directly gasometrically in the Warburg apparatus. Perfusion with sodium cyanide (12 experiments) led to an inhibition of bile secretion to the extent of 32-80%, mean value – 63% (see figure). Further perfusion with fresh saline did not reverse the inhibition; bile secretion remained on the low level or, sometimes, decreased further. Similar observations were made by S. Ia. Rapoport and K. A. Gerchikova [3], who found that asphyxia in situ caused a sharp drop, or even cessation, of the bile flow. Interference with tissue respiration was thus shown to depress bile secretion severely.



The effect of enzyme inhibitors on bile secretion by the isolated cat liver (in ml per 30 min).

Glycolysis in livers was depressed by perfusion of the isolated organs with monoiodoacetic acid or with sodium fluoride (20 experiments). Sodium fluoride inhibited bile formation in the isolated liver to the extent of 16-60%, mean 38% (see figure). Washing with physiological saline caused further inhibition of the bile flow. Similar results were obtained with monoiodoacetic acid. In in situ experiments, using rabbits, it was found that monoiodoacetic acid caused a mean inhibition of 42% of the bile secretion, when administered in amounts 30 mg/kg. Thus, in isolated livers, and in experiments in situ, the bile secretion was found to be inhibited when either tissue respiration or glycolysis were interfered with.

According to established concepts, the energy of glycolysis and respiration is not utilized directly, but is channelled for the resynthesis of ATP; the splitting of this compound then provides the necessary energy in the various biosynthetic reactions. ATP resynthesis in the liver may take place through the coupling of phosphorylation with either respiration or glycolysis. In order to distinguish between the respective contribution of respiration and glycolysis toward the energetic balance in the synthesis of the high transfer potential groups of ATP, the respective values of respiratory and glycolytic phosphorylation were determined using rabbit liver homogenates (see table).

The P-uptake values found were, under aerobic conditions, 0.66 mg P_0 per test, under anaerobic conditions – 0.11 mg. The P/O ratios were 1.57. The present data are thus in agreement with existing opinion [2, 4], that oxidative reactions in the liver provide the main source of energy, through the formation of high transfer potential phosphate groups of ATP. This gains further support from the present experiments dealing with the levels of ATP after the introduction of cyanide and fluoride. As a rule, liver transfusion with cyanide caused considerable fall in ATP concentration (mean, from 12.3 to 5.1 mg%) while fluoride had virtually no effect on the liver ATP levels.

In another series, involving 38 experiments, attempts were made, with the use of 2,4-dinitrophenol and sodium arsenate, to elucidate the energetic role of aerobic and anaerobic phosphorylation in the process of bile formation and its mechanism. As is known, 2,4-dinitrophenol causes the uncoupling of oxidation from the resynthesis of ATP, thus rendering the former process energetically useless, while in the presence of arsenate, phosphotriose oxidation may take place without ADP phosphorylation, i.e., there takes place the uncoupling between the glycolytic oxidation–reduction processes and ADP rephosphorylation [1].

If anaerobic phosphorylation be energetically essential for the process of bile secretion, then arsenate poisoning should cause the depression of the phenomenon under investigation, in the same way as did monoiodoacetic acid and fluoride. However, experimental evidence (involving perfusion of the isolated liver with arsenate) showed that arsenate did not affect the rate of bile secretion. An entirely different result was obtained with dinitrophenol: bile secretion fell by 24-78% (mean – 50%; see figure). The observation thus showed that bile formation depended energetically on oxidative phosphorylation.

Expt. No.	Inorganic phosphorus esterified mg		Oxygen consumption in mm^3 per 40 min	P/O ratio (anaerobic phosphorylation excluded)
	N_2	O_2		
39	—	0,68	200,3	1,23
40	—	0,73	142,4	1,86
41	0,12	0,67	137,5	1,76
42	0,11	0,65	157,5	1,49
43	0,10	0,72	177,1	1,47
44	—	0,78	169,5	1,67
45	—	0,52	111,0	1,70
46	0,13	0,70	166,9	1,52
47	0,10	0,57	144,2	1,43
48	0,09	0,65	156,9	1,50
49	0,10	0,67	141,4	1,71
50	0,11	0,55	138,1	1,45
Mean	0,11	0,66	153,6	1,57

The evidence presented above allows the conclusion that tissue respiration, together with the coupled phosphorylation process, provide the main energy source for bile secretion. Glycolytic processes affect some specific reactions of bile formation, rather than its energetics. The latter aspect requires further investigation.

SUMMARY

Experiments were performed on animals with fistula of the common bile duct and on isolated liver with fistula of the gall bladder. It was established with the aid of enzyme poisons (sodium cyanide, sodium fluoride, monoiodacetic acid, 2,4-dinitrophenol, sodium arsenate) that tissue respiration of liver and oxidative phosphorylation connected with it are the main origins of energy in bile secretion.

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THE THERMAL EFFECT OF THE SUPERHIGH FREQUENCY ELECTROMAGNETIC FIELD

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(Received November 2, 1957. Presented by Active Member AMN SSSR V. V. Parin)

As a result of the widespread introduction of radio engineering into the national economy, the human organism has begun to be subjected, not infrequently, to the action of a new stimulus to which it is not accustomed—radio waves. These occupy that portion of the electromagnetic spectrum having waves from millimeters to tens of thousands of meters in length. The part where the waves are shortest— the super-high-frequency region (SHF), covering the band from millimeters to tens of centimeters— has been the least studied. One of the differences between the SHF field and the UHF field is the ability of the former to be concentrated in a directed beam, which can exert its effect at a given distance. The most pronounced effect of the SHF field on the organism is its thermal effect.

Temperature changes in the organism, under the influence of UHF as well as of SHF, have been studied before [1, 2, 3, 4, 5, etc.] but without taking the intensity of the action into account, so that it is difficult to compare the results so obtained. Several investigations [6, 7, 8, etc.], and also our own data (1955-1957) indicate that the temperature increase under the influence of these waves depends on their length, on the time and intensity of irradiation, and on electrical and thermal constants.

In the present study, data are presented on the characteristics of the temperature rise in certain types of animals under the influence of the SHF field which will be of definite interest for the clarification of the mechanism of its thermal action and for working out the doses for the use of microwaves as a therapeutic agent, and also for the solution of certain professional problems of preventive medicine.

To accomplish this task, thermometry was carried out at points directly within the SHF field on dogs, cats, rabbits, rats, and frogs (170 animals in all). Temperature was recorded with the aid of an EPP-09 potentiometer, with Chromel-Copel needle thermocouples.

In this work, allowance was made for the special features of the arrangement of thermocouples perpendicularly to the direction of the electric field vector E [4]. Instrumental measurements of radiation intensity were carried out by engineers.

EXPERIMENTAL RESULTS

In an investigation of the thermal effect of the SHF field at an energy flux of 0.1-0.3 watt/cm^2, the rectal temperature increased more markedly and more abruptly in small animals than in large ones (Fig. 1).

In small animals, the rectal temperature began to rise at the beginning of irradiation; in larger animals, after 1-5 minutes or more, depending on their size.

The character of the warming curve also depended on the energy flux; with reduction in radiation intensity, a decrease in the temperature rise was observed. In the rat, for example, at an energy flux of 0.1 watt/cm^2, the magnitude of the increase in rectal temperature was less than in a field of greater intensity (Fig. 2). The time required for the temperature to return to the initial level after irradiation is stopped also depends on the

dimensions of the animal. The temperature curves followed the law of ascending (in warming) and descending (in cooling) exponentials.

When different animals were irradiated in fields of different intensity, the survival time was not the same. Thus, at an energy flux of 0.3-0.2 watt/cm^2, the rats died after 12 minutes, on the average; rabbits, after 25-35 minutes; cats, after 45-60 minutes. Under these conditions, a large dog still remained alive even after 2 hours.

At lower intensities of radiation, the survival time of the animals was correspondingly prolonged.

In all types of experimental animals, no rise in rectal temperature was observed at an energy flux of 0.01 watt/cm^2 (Table 1).

From data on the distribution of heating according to depth, one can form an idea of the mechanism of action of waves of different lengths.

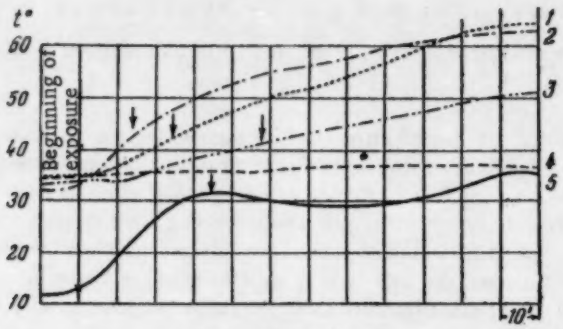

Fig. 1. Variation in rectal temperature of different animals. 1) Rabbit; 2) rat; 3) cat; 4) dog; 5) frog.

In dogs, cats, rabbits, and rats, the greatest temperature rise was observed under the skin; for example, at the time of death of a rat in an intense field (energy flux 0.3 watt/cm^2), the subcutaneous temperature was 46-50°. Thus, the temperature rose 10-14°. The increase in temperature began simultaneously with irradiation and was considerably greater than the rise in rectal temperature. In dogs, the temperature under the skin rose 3.5° (while the rectal temperature rose 1.5°).

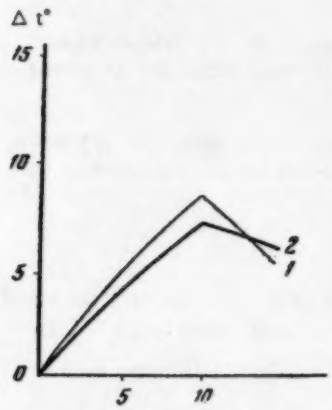

Fig. 2. Curves of temperature increase in the rat (potentiometric data). 1) Subcutaneous temperature; 2) rectal temperature.

With reduction in radiation intensity the subcutaneous warming decreased (see Fig. 2); even in rats, it was not observed at an energy flux of 0.01 watt/cm^2.

Deep in the thigh muscle and in the brain of the rabbit at a depth of 1-1.5 cm, during irradiation in an SHF field (energy flux 0.3 watt/cm^2) the rise in temperature did not begin immediately, but 1-3 minutes after the beginning of irradiation, and reached 7.6° (Table 2).

Thus with equal energy fluxes and irradiation time, the energy entering per unit weight is less in the large and greater in the small animal. In large animals, more superficial warming was observed. This is related to the fact that the vital organs in larger animals are more deeply situated and are consequently less strongly heated than in small animals. This also determines the degree of overheating, in a certain measure, and the times of death. The total heating of the body of the animal is determined by the wavelength of the SHF field; on account of heat conduction and circulation of the blood, heat is carried from parts of the body heated more to

those heated less. The circulation simultaneously plays the role both of a warming and of a cooling factor. In view of the fact that under irradiation in an SHF field warming takes place unequally, the role of the circulation, along with heat conduction, probably predetermines the ultimate magnitude of the general overheating, and, therefore, the death of small animals as well.

TABLE 1

Dependence of Rectal Temperature in Various Kinds of Animals on Energy Flux and Exposure Time

Energy flux in watt/cm²	Dog	Cat		Rabbit		Rat	
	temperature rise after 2 hours	exposure time in minutes	temperature rise	exposure time in minutes	temperature rise	exposure time in minutes	temperature rise
0,2—0,3	1,5°	60	7,0°	25	7,5°	12	10,0°
0,05—0,1	0,5°	120	6,0°	120	6,6°	19	7,8°
0,02—0,04	0°	120	1,2°	120	1,5°	120	3,4°
0,01	0°	120	0	120	0°	120	0

One of the important factors causing a difference in the extent of temperature increase in individual types of animals is the efficiency of temperature regulation by the nervous system. Thus, when exposed to an SHF field (energy flux 0.05 watt/cm²) that will cause the death of a rabbit after 1-1 1/2 hours, with 6° of overheating, a cat of the same weight dies after a 2-hour exposure, and a dog (of approximately the same weight) generally does not die.

TABLE 2

Dependence of Temperature in Rabbit Organs on Energy Flux, and Survival Time in Exposure to SHF Field

Energy flux in watt/cm²	Temperature				Exposure time in minutes
	rectal	in muscle	in brain	subcutaneous	
0,2—0,3	7,5°	7,6°	7,6°	12,5°	35 (died)
0,1—0,05	6,6°	7,0°	7,1°	8,1°	115 »
0,02—0,04	1,5°	1,5°	—	2,5°	420 (survived)
0,01	—	—	—	—	420 » »

In spite of the fact that dogs can be irradiated longer than other animals, exposure cannot be considered harmless even for them. Maintenance of body temperature at an approximately normal level under such conditions requires considerable effort on the part of the neuroregulatory apparatus, which, under prolonged and repeated exposures, may be not without consequences for the organism. Evidence for this is found in our investigations of cardiac activity by the electrocardiographic method [8], and the studies of other authors.

The data obtained permit us to divide the effects of the SHF field into thermal and extrathermal effects. The thermal action in turn can be divided (for small animals, including cats) into lethal effects, leading to death of the animals after a certain period of exposure (up to 2 hours at an energy flux of 0.05 watt/cm²), and sublethal effects, which do not cause death of the animals even after a longer period and are at a lower radiation intensity.

We regard as "extrathermal conditions" the maximum energy flux at which prolonged exposure (more than 2 hours) causes neither general nor local increase in temperature, as far as can be detected by methods available

to us. This limit occurs at an energy flux of 0.01 watt/cm^2; respiration, blood pressure, and cardiac rhythm are not noticeably altered under these conditions [6].

This limit varies depending on the size of the animal: for large animals (dog) it lies within the limits stated above; for small animals it is somewhat lower.

SUMMARY

Rectal and subcutaneous temperatures were studied in experiments on 170 animals—dogs, cats, rabbits, rats, and frogs—by the method of continuous thermometry in super-high-frequency electromagnetic fields of different intensities. In addition, thermometry was carried out in muscles and brain of rabbits. The survival time of various animals was determined, as well as the temperature increase. The characteristics of heating and cooling were investigated. The temperature curves followed the law of ascending and descending exponentials in heating and cooling, respectively. The heating effect was greatest in the subcutaneous tissue, less in the brain and deep in the muscles of the thigh, and even less in the rectum. The thermal effect, in the super-high-frequency field, depends on the energy flux, the exposure time, and the size and species of the animal. The lower limit of the "thermal conditions" which cause the death of small animals, including cats (lethal effect), is 0.05 watt/cm^2. The lower limit of the "thermal conditions", at which there is no heating effect on tissues, is 0.01 watt/cm^2.

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PHARMACOLOGY

EFFECT OF PROCAINE ON TOLERANCE OF WHITE RATS TO HIGH ALTITUDES

PART II. EFFECT OF p-AMINOBENZOIC ACID

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We showed in our previous communication [7] that administration of procaine raised the tolerance of animals to high altitude conditions. The view was expressed that procaine not only exerts a local blocking action on nerve endings of interoceptors, but also has a generalized effect on the central nervous system. It was thought, in this connection, to be desirable to investigate the effects on organisms exposed to such conditionsof substances having a structural resemblance to procaine.

Procaine is the hydrochloride of the diethylaminoethyl ester of p-aminobenzoic acid. It is readily broken down in the organism to p-aminobenzoic acid and diethylaminoethanol [3]. p-Aminobenzoic acid is a vitamin, which according to the findings of N. I. Leporskii and T. T. Karakulina [4] has a similar physiological action on the central nervous system to procaine. Moreover, restoration of the color of grey hair is observed with large doses of procaine, and this is characteristic of the action of p-aminobenzoic acid, as a vitamin.

Thus procaine and p-aminobenzoic acid are similar in their chemical structure and in their action on the organism.

EXPERIMENTAL METHODS

We applied the same methods to the study of the action of p-aminobenzoic acid as we used previously with procaine. The animal material used consisted of adult white rats. Some of the rats were housed in separate cages, and were fed the same diet as the control group, with the addition of 200 mg of p-aminobenzoic acid per 100 g of food. This dosage level has been reported in the literature [5] as not having toxic effects. Both groups of animals were placed in a pressure chamber after the lapse of a day, and the pressure was lowered to that of an altitude of 11,000 m, at a rate of 30 m per second, and maintained at that level for 10 minutes. In all, we used 114 rats, 58 of which received p-aminobenzoic acid, and 56 served as controls.

The results obtained are presented in the table.

Effect of p-Aminobenzoic Acid on the Survival Rate of White Rats at a Barometric Pressure Corresponding to an Altitude of 11,000 m

Experimental conditions	Number of animals	Number surviving	
		in absolute figures	in %
Rats not receiving p-aminobenzoic acid (controls)	56	23	41
Rats receiving p-aminobenzoic acid	58	48	82

The results show that the survival rate of animals receiving p-aminobenzoic acid is double that of the controls, at an altitude of 11,000 m. This finding is of some practical importance, since it shows that p-aminobenzoic acid can be given, together with other vitamins, for the purpose of raising the tolerance of organisms to oxygen lack [2, 6].

SUMMARY

It was experimentally established that para-aminobenzoic acid increases the survival of rats raised to high altitudes. Experiments performed on 114 rats with the addition of para-aminobenzoic acid to their food (200 mg per 100 g) demonstrated that the mortality rate of these animals decreased by half. A conclusion was drawn that para-aminobenzoic acid increases the resistance of the body to oxygen insufficiency.

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**Original Russian pagination. See C. B. Translation.

EFFECT OF NITROUS OXIDE ON "SPONTANEOUS" SALIVATION IN DOGS

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Narcotics (alcohol and chloroform) have been shown by S. I. Potekhin [2] and by E. Z. Pushkareva and E. A. Bellavskaia [3] to cause "spontaneous" secretion of saliva in dogs. While studying the effects of nitrous oxide on conditioned reflexes we also observed "spontaneous" salivation, with masticatory movements, in dogs inhaling the gas.

The wide and varied use made of nitrous oxide in medical practice necessitates the detailed study of its effects on the various functions of the organism. The present research is devoted to the study of the nature of "spontaneous" secretion of saliva during inhalation of nitrous oxide, and to the elucidation of its mechanism.

EXPERIMENTAL METHODS

The experiments were performed in a conditioned reflex chamber, on 4 tracheotomized dogs, with a fistula of the parotid gland duct. The animals were prepared for experiment according to the classical Pavlovian procedure, described in N. A. Podkopaev's manual [1]. Mastication, respiration, and secretion of saliva were recorded on a smoked kymograph sheet during the experiments.

Nitrous oxide – oxygen mixtures, containing 20, 40, 60, and 80% of nitrous oxide, were administered without the knowledge of the animals (bypassing the upper respiratory passages), through a special intratracheal cannula, inserted into a tracheotomy incision. The dogs inhaled that mixture from a Douglas bag, or from a gas – oxygen apparatus. This contained meters, permitting of the regulation of the composition and amount of the mixture. Additionally, the composition of the mixture was checked throughout the experiments by means of P. M. Starkov's thermal gas analysis method [6]. In all, we performed 83 experiments with the use of nitrous oxide, and 12 control experiments. In these, the animals inhaled air or oxygen.

EXPERIMENTAL RESULTS

The phenomena of "spontaneous" salivation and mastication were first seen in the dogs Valetka and Mart, using 40% nitrous oxide mixture. In these dogs, we started the flow of gas after the conditioned stimulus had been reinforced by meat-biscuit powder, and after a control test of the conditioned reflex, when the salivation and mastication associated with the act of feeding had entirely ceased. After $\frac{1}{2}$ – 2 minutes of inhalation of 40% nitrous oxide we observed the onset of "spontaneous" salivation and of masticatory movements. The secretion of saliva amounted to 1 – 4 drops per 30 seconds, and the frequency of the masticatory movements to 1 – 5 (observations from 8 experiments). Similar effects were not seen with 20% nitrous oxide mixtures. Taking into account the weakening effect of nitrous oxide on the process of internal inhibition, it might be supposed that the secretion of saliva and the masticatory movements observed during inhalation of 40% nitrous oxide develop as a result of release of inhibition of the temporal conditioned reflex.

In order to check these observations, and for their further study, we repeated the experiments with two other dogs, Laska and Sedoi, which had never been fed under the conditions of the experiment. The experimental procedure was as follows. After preparation, the dogs were left in the stand. We registered the background levels

of salivation and masticatory movements for 5-7 minutes; usually 1 drop of saliva was secreted during 3½ minutes, and there were no masticatory movements. Inhalation of nitrous oxide was then started.

Masticatory movements were seen within 2-3 minutes of inhalation of 40% nitrous oxide. Their frequency varied from 1 to 9 in 30 seconds for Laska (data from 5 experiments) and from 1-12 for Sedoi (data from 6 experiments). "Spontaneous" salivation was not seen for either dog, except for two experiments with Sedoi, in which single drops appeared.

The appearance of "spontaneous" salivation in Valetka and Mart, but its absence in Laska and Sedoi, evidently depended on the different alimentary stimulability of the dogs during the experiment. The alimentary center of Valetka and Mart was highly receptive to stimulation, as a result of the experimental conditions, which always involved the giving of food, with reinforcement of the conditioned stimulus by presentation of meat–biscuit powder 3-5 minutes before administration of nitrous oxide. No such factors were active in the other two dogs, raising their receptivity to alimentary stimuli.

Our further experiments showed that the above interpretation was justified, as after repeated feeding in the harness "spontaneous" salivation was seen in Laska and Sedoi after administration of 40% nitrous oxide, just as for Valetka and Mart. Raising the nitrous oxide concentration of the mixtures to 60 and 80% caused a sharp increase in "spontaneous" salivation and mastication.

At the same time, administration of 60 and 80% nitrous oxide mixtures led to rapid habituation of the animals, shown by the falling off of the secretory and masticatory effects when the experiment was repeated using the same concentration of gas (Fig. 1).

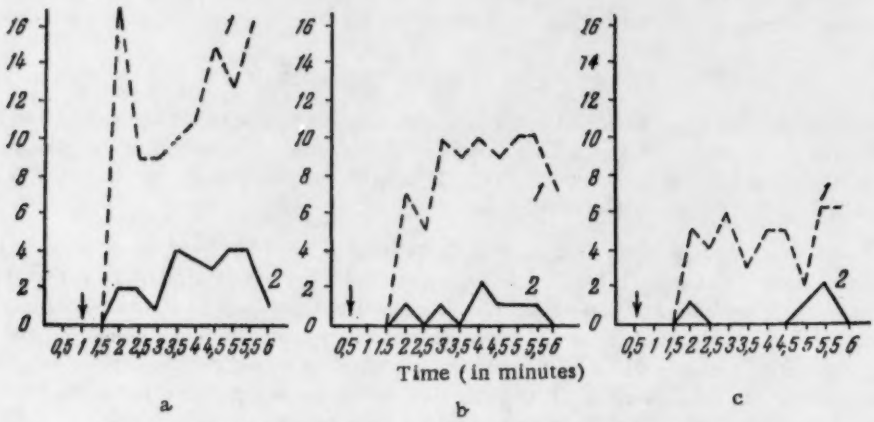

Fig. 1. Recordings of "spontaneous" salivation and masticatory movements in the dog Sedoi during administration of 80% nitrous oxide mixture. a) Experiment No. 23, of February 20, 1954; b) Experiment No. 24, of February 22, 1954; c) Experiment No. 25, of February 23, 1954. 1) Frequency of masticatory movements, 2) amount of "spontaneous" secretion of saliva; the arrows indicate the moment when nitrous oxide was given; the abscissae are time in minutes, and the ordinates express drops of saliva and frequency of masticatory movements.

Our observations show that nitrous oxide, at concentrations of 40, 60, and 80% causes "spontaneous" secretion of saliva and masticatory movements. The intensity of these effects depended on the concentration of nitrous oxide, on the receptivity of the animal to alimentary stimuli, and also apparently on the individual type of higher nervous activity of the given animal.

What is the mechanism of development of "spontaneous" salivation and of masticatory activity during administration of nitrous oxide? V. K. Fedorov [7] ascribed the increase in unconditioned secretion of saliva during

the action of chloral hydrate to the heightened excitability of subcortical centers, following their release from inhibitory control by the cortex. Such an interpretation is supported by the observations of A. I. Smirnov [5] and of T. P. Rolich [4], who found that "spontaneous" salivation appeared in 40-50% of dogs after removal of the cerebral cortex. According to these authors, decerebration raises the sensitivity of the center controlling secretion of saliva to chemical stimuli present in the blood stream.

"Spontaneous" salivation and masticatory movements made their appearance at nitrous oxide concentrations (40, 60, and 80%) at which cortical processes were considerably depressed. It might, in this connection, be supposed that the secretion of saliva and the masticatory movements were due to the enhanced activity of the subcortical alimentary center.

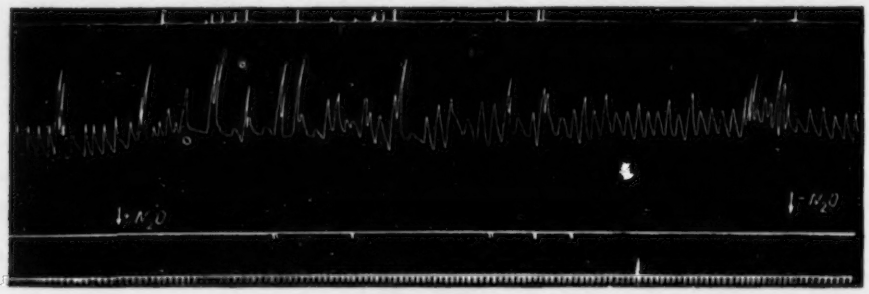

Fig. 2. Salivation and masticatory movements observed when nitrous oxide was passed through the upper respiratory passages of the dog Sedoi (Experiment No. 27, March 8, 1954). Explanation of tracings (from above down): recordings of masticatory movements, recording of respiration, recordings of drops of saliva, time marker (3 seconds).

S. I. Potekhin [2] regarded "spontaneous" salivation of animals given alcohol as being a reflex from the nasal mucosa to stimulation by alcohol.

Nitrous oxide is known to diffuse rapidly and readily through animal tissues. P. M. Starkov [6] showed that it is eliminated through the skin, exposed muscles, and other tissues.

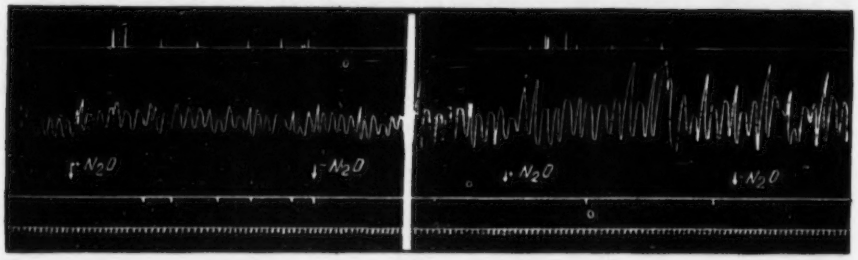

Fig. 3. Salivation and masticatory movements observed during passage of nitrous oxide through the upper respiratory passages of the dog Laska, in Experiments Nos. 52 and 53 on January 28 and 31, 1955. The fall in the magnitude of the effects with repetition of the test is evident. Explanation of tracings as in Fig. 2.

We therefore thought it possible that nitrous oxide might be eliminated from the body through the mucous membranes of the upper respiratory passages, where it might directly stimulate receptors, thus causing reflex "spontaneous" secretion of saliva and masticatory movements.

The following experiments were designed to test this supposition. We inserted two intratracheal tubes into dogs, one being directed toward the lungs, and the other towards the larynx. The dogs breathed in air from the first tube, while pure nitrous oxide was passed into the other, at a rate of 85-250 cc per minute. These experiments were performed on Sedoi, which had had 12 previous exposures to nitrous oxide.

"Spontaneous" salivation and masticatory movements were observed while nitrous oxide was being passed over the mucosa of the upper respiratory passages. The results are shown in Fig. 2.

Repetition of this experiment showed that the magnitude of the response to flow of nitrous oxide through the upper respiratory passages fell from experiment to experiment, salivation no longer appearing during the third test, and mastication during the fifth test.

It seems from our results that the "spontaneous" salivation and the masticatory movements observed in response to stimulation of the upper respiratory passages with nitrous oxide resembled the secretory reflex observed by S. I. Potekhin in response to alcohol, which he considered to be a natural conditioned reflex. In order to elucidate the nature of the reflex we repeated the experiments with the dogs Laska and Topsi, which had not before been exposed to nitrous oxide. These dogs gave no salivary or masticatory response to flow of gas or air through the upper respiratory passages. This result shows that the reflex is not an unconditioned one. Laska was then exposed 12 times to the action of nitrous oxide (60 and 80%). When gas was then passed through the upper respiratory passages salivation and masticatory movements appeared (Fig. 3). As can be seen from Fig. 3 a smaller response resulted from a second passage of nitrous oxide through the upper respiratory passages. No response was seen when the test was repeated a third time.

Our experimental results show that the secretion of saliva and the masticatory movements seen when the upper respiratory passages are exposed to the action of nitrous oxide constitute a natural conditioned reflex, which is elaborated by repeated exposure of the animal to the gas, and which subsequently undergoes extinction.

SUMMARY

Inhalation of nitrous oxide – oxygen mixtures containing not less than 40% of nitrous oxide causes "spontaneous" salivation and masticatory movements in dogs. The magnitude of the response varies with the concentration of nitrous oxide, with the sensitivity to stimulation of the alimentary center, and with the previous conditioning of the animals. The effect is due mainly to strengthening of the function of the subcortical alimentary center, resulting from removal of cortical inhibition. After numerous exposures of the animals to inhalation of nitrous oxide the effects may be elicited by passing the gas through the upper respiratory passages only. This effect is mediated by natural conditioned reflex, which is elaborated by repeated exposure to the gas, and which suffers extinction with further repetition.

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MICROBIOLOGY AND IMMUNITY

THE STUDY OF ANTIGENIC PROPERTIES OF CERTAIN CORPUSCULAR AND SOLUBLE FRACTIONS ISOLATED FROM TUMORS OF INBRED MICE

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The investigations of L. A. Zil'ber and his co-workers [2] established the presence of specific antigens in the tissues of malignant tumors.

The search for antibodies for a specific antigen, the development of methods of immunization against recurrence of tumors and other problems in tumor immunology required the isolation of the specific tumor antigen in a purer and more concentrated form. One of the ways of solving this problem was the isolation of different fractions from the tumor cell and the study of their antigenic properties.

L. A. Zil'ber, N. V. Nartsissov and G. I. Abelev [2] demonstrated that the specific antigen of the rat tumor-M-1, which possesses serological activity, is associated primarily with the fraction of mitochondria and microsome and with one of the globulin fractions isolated from the hyaloplasm. In these investigations they used tumor induced and transplanted in animals of impure breed which could be distinguished from the tissues of its host by its antigenic composition. In addition, N. V. Nartsissov and N. V. Abelev demonstrated the presence of a specific antigen in experiments with primary rat tumors induced with carcinogens.

This project carried out at L. A. Zil'ber's suggestion was concerned with the distribution of specific antigens in different fractions of tumors transplanted onto animals of pure breed and therefore not having isoantigenic differences with the tissues of these animals – carriers of the tumor.

EXPERIMENTAL METHOD

As material we used two tumor strains transplanted onto mice of pure line C_3HA: strain of breast adenocarcinoma isolated by us from a spontaneous tumor which arose in mouse C_3HA and strain of hepatoma resulting from a tumor induced by ortho-aminoazotoluene (V. I. Gel'shtein) in the laboratory directed by L. M. Shabad. Hepatoma is especially suitable in view of the fact that the liver of healthy mice of the same line can serve as an adequate control for this tumor.

For comparison of antigens of tumor and normal tissues we employed the anaphylactic reaction with desensitization which was also used in conducting basic investigations in the detection of specific antigens in tumors. Tumor tissue was subjected to fractionation by the method of differential centrifugation and separation described in the work of N. V. Abelev and G. S. Bezverkhii [1]. Tumor tissue in a tenfold volume of 8% solution of saccharose was reduced to fragments by a homogenator in 7 minutes. Nuclei and remnants of undestroyed tissue were removed from the homogenate by triple centrifugation at 1,500 revolutions per minute. The supernatant fluid was diluted with an equal volume of saccharose and passed through separator ASG-1 with a pulley giving a maximum speed of 16,000 revolutions per minute. Microscopic control showed that at this speed mitochondria and a considerable portion of microsomes are precipitated out. The precipitate of mitochondria and microsomes is removed from the separator, washed in an 8% solution of saccharose, dissolved in distilled water of pH = 7.6 and used in the investigations.

After separation, the supernatant fluid representing hyaloplasm with an admixture of microsomes was dialyzed to remove the saccharose after which the protein fraction, obtained by salting out the soluble cytoplasmic proteins with ammonium sulfate at 33% saturation in the cold, was removed. Inasmuch as this fraction is obtained by a method analogous to the one for obtaining serum globulins, we assume that with this method we isolate proteins of the globulin type and for this reason name it the globulin fraction.

Anaphylactic reaction with desensitization was used to test the fractions isolated after their solution. Three groups of pigs were sensitized hypodermically with approximately equal amounts of protein (about 4 mg) from each of the fractions of cancerous tissue of mouse breast: the original extract of tumor (supernatant fluid after the removal of nuclei), the mitochondria – microsome fraction and the globulin fraction.

The experiment was performed a month later. The pigs of each group were sensitized with intravenous injections of the corresponding fraction prepared from a mixture of organs of healthy mice of the same line. All pigs responded to this injection with an anaphylactic shock which indicated the presence of normal tissue proteins in the fractions isolated from the tumors. Antigens from normal tissues were injected repeatedly with the purpose of producing desensitization of the pigs to them. After the animals no longer reacted to the injection of a definite dose of antigens from normal tissues (usual dose exceeded the sensitizing dose by $1\frac{1}{2}$ or 2 times), that fraction from tumor tissue to which they were sensitized was administered in a dose not exceeding that for testing the completeness of desensitization.

EXPERIMENTAL RESULTS

In Table 1 are presented the results of such an experiment. The majority of pigs responded to the resolving injection of the antigen studied with an anaphylactic shock. From this the conclusion was drawn that not only the original extract from cancerous mouse breast, but the tested fractions as well – mitochondria – microsomes and globulin – contain a specific antigen distinct from antigens of normal tissues.

In Table 2 is presented data of a similar experiment in which fractions isolated from mouse hepatoma were used for sensitization and for the resolving injection. Pigs sensitized with the original extract and with fractions of mitochondria – microsomes and globulin from the hepatoma and completely desensitized with the same fractions from the liver of healthy mice of the same line C_3HA onto which the hepatoma is transplanted, responded with anaphylactic shock when fractions used for sensitization were injected. Therefore, the original extract of mouse hepatoma as well as fractions of mitochondria – microsomes and globulin contain a specific antigen.

Besides the above-described fractions, those isolated from the same tumors by the method of methanol precipitation were also studied employing anaphylactic reaction with desensitization [5]. As is known, acid albumins of nucleoprotein nature are isolated by this method which is also used to isolate viral proteins.

It became essential to isolate such a fraction from both tumors (one of which was viral) and to determine the antigenic properties of the preparations obtained.

In Table 3 are presented the results of the experiment which shows that specific antigen is present in the methanol precipitate from cancerous breast. In this experiment, pigs were sensitized with the methanol precipitate from spontaneous or transplanted breast adenocarcinoma in mice. Following desensitization with methanol precipitate from a mixture of organs of healthy mice of the same line, and in a number of cases – from the breasts of these mice, the pigs responded with anaphylactic shock when methanol precipitate from cancerous breasts was injected.

In the second part of Table 3 are presented the results of the experiment in which pigs sensitized with methanol precipitate from the liver of healthy mice of the same line responded with anaphylactic shock to the injection of methanol precipitate from hepatoma. Therefore, the methanol precipitate from hepatoma of a mouse in an anaphylactic reaction with desensitization also contains a specific antigen, distinct from antigens in methanol precipitates from the liver of healthy mice of line C_3HA.

Thus, proteins of cellular organoids – mitochondria and microsomes and globulin fraction of hyaloplasm proteins, as well as acid proteins precipitated in methanol precipitate, – contain specific antigen.

Earlier we showed [3] that nucleoprotein fraction isolated from hepatoma contains specific tumor antigen.

TABLE 1

The Study of Fractions from Cancerous Tumor Tissue of the Breast of Mice in a State of Anaphylactic Reaction with Desensitization•

Pig No.	Sensitization		Desensitization			Testing for completeness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
17	Original extract from	4.5	Original extract	1.2	++	Original extract	5.0	–	Original extract	4.8	+
19	breast cancer of mice	4.5	from a mixture	1.2	+	from a mixture	5.6	–	from breast of	4.5	++
20	of line C_3HA	4.5	of organs of healthy mice of line C_3HA	1.2	++	of organs of healthy mice of line C_3HA	6.2	–	mice of line C_3HA	5.7	+++
123	Mitochondria and	4.4	Mitochondria and	3.7	+	Mitochondria	7.5	–	Mitochondria and	7.2	±
128	microsome fraction	4.4	microsome fraction from a mixture of organs of healthy mice of line C_3HA	2.5	++	and microsome	8.8	–	microsome fraction from breast cancer of mice of line C_3HA	8.5	++
132	from breast cancer	4.4		5.0	++	fraction from a	10.0	–		9.1	+
133	of mice	4.4		2.0	+	mixture of organs	6.5	–		6.5	+
136	of line	4.4		2.0	+	of healthy mice	6.5	–		6.5	–
137	C_3HA	4.4		3.3	+++	of line C_3HA	6.5	–		6.5	++
115	Globulin fraction	3.9	Globulin fraction	1.1	++	Globulin fraction	4.4	–	Globulin fraction	4.3	+
116	from breast cancer	3.9	from a mixture of	1.5	++	from a mixture	5.2	–	from breast cancer of mice of line C_3HA	5.1	–
118	of mice of line	3.9	organs of healthy	1.6	+	of organs of	4.7	–		5.1	+++
119	C_3HA	3.9	mice of line C_3HA	2.3	+	healthy mice of line C_3HA	5.5	–		5.1	++

Designations: – absence of reaction; + temperature drop, momentary scratching of the nose, sneezing; + + temperature fall, strong scratching, sneezing, ruffling of fur, cough; +++ same, but more marked; elimination of urine and feces; ++++ convulsive jumping, convulsions terminating in death of the animal.

•In this and the following tables only the results of the first desensitization and the testing for completeness of desensitization are indicated; the intermediate stages of desensitization are omitted.

TABLE 2

A Study of Fractions from Hematoma of Mice in a State of Anaphylactic Reaction with Desensitization

Pig No.	Sensitization		Desensitization			Testing for completeness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
4	Original	4	Original extract	1.3	+++	Original extract from	7.9	–	Original	7.4	+++
6	extract from	4	from liver of	1.3	+	liver of healthy mice	7.9	–	extract from	5.7	±
7	hepatoma	4	healthy mice of line C_3HA	1.3	++	of line C_3HA	9.2	–	hepatoma	8.2	+++
11	Mitochondria and	4.2	Mitochondria and	1.4	+	Mitochondria and	8.4	–	Mitochondria	7.0	+
12	microsome fraction	4.2	microsome fraction	2.8	+	microsome fraction	8.4	–	and micro-	8.4	++
45	from hepatoma	4.2	from liver	2.8	±	of liver of	8.4	–	some fraction	8.4	++
9		4.2	of healthy mice	5.6	±	healthy mice	1.2	–	from hepa-	9.	–
46		4.2	of line C_3HA	2.8	++	of line C_3HA	9.8	–	toma		±
40	Globulin	4.2	Globulin fraction	1.5	+++	Globulin fraction	7.1	–	Globulin	4.8	±
43	fraction	4.2	from liver of	0.5	+	from liver of	2.7	–	fraction	2.4	+
44	from hepatoma	4.2	healthy mice of line C_3HA	1.5	+++	healthy mice of line C_3HA	3.0	–	from hepatoma	3.0	+

Designations same as in Table 1.

TABLE 3

Anaphylactic Reaction with Desensitization with Methanol Precipitate from Breast Cancer and Hepatoma of Mice

Pig No.	Sensitization		Desensitization			Testing for completness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
120	Methanol precipi-	4.3	Methanol precipitate	2.9	+	Methanol precipitate	5.8	−	Methanol pre-	4.5	++
121	tate from trans-	4.3	from a mixture of	2.2	++	from a mixture of	5.8	−	cipitate from	5.4	±
125	planted	4.3	organs of mice of	1.4	−	organs of healthy	5.4	−	transplanted	5.4	+
126	breast	4.3	line C_3HA	2.2	++	mice of line C_3HA	5.8	−	breast cancer	5.4	−
131	cancer	4.3		2.2	−		5.8	−		5.4	++
168	From spontaneous	4	From breast of mice	0.8	+		4.6	−	From spon-	4.3	+++
169	breast cancer	4	of line C_3HA	1.6	++		4.6	−	taneous breast cancer	4.3	±
173	The same	4	From breast of mice	2.2	++	From breast of mice	4.5	−		4.3	−
165	"	4	of line C_3HA	1.3	+++	of line C_3HA	3.1	−	From trans-	3.1	++
									planted breast cancer		
167	"	4	From a mix-	1.3	−	From a mixture of	6.3	−	The same	6.2	+
170	"	4	ture of organs	1.9	++++	organs of healthy	8.8	−	"	7.4	+
172	"	4	of healthy	1.9	−	mice of line	6.3	−	"	6.2	+
174	"	4	mice of line	3.2	++	C_3HA	6.3	−	"	6.2	+
169	"	4	C_3HA	0.6	++		6.3	−	"	6.2	++
173	"	4		0.6	++				"		
135	Methanol	4.2	Methanol precipitate	1.4	+	Methanol precipitate	4.2	−	Methanol pre-	4.4	+
140	precipitate from	4.2	from liver of	1.8	+	from liver of	5.4	−	cipitate from	5.5	+
142	transplanted	4.2	healthy mice of	3.6	+	healthy mice of	6.0	−	hepatoma	6.0	++
143	breast cancer	4.2	line C_3HA		·	line C_3HA	·	·		6.5	++
214		2.9		0.9	+		6.2	−		6.0	+
215		2.9		0.9	+		6.2	−		6.0	+
219		2.9		0.9	+		6.2	−		6.0	+
222		2.9		0.9	+		6.2	−		6.0	+

Designations same as in Table 1. Dot indicates that no injection was given.

Naturally, the question arises: how to correlate the data of the previous experiment dealing with antigenic activity of the nucleoprotein fraction with the data dealing with antigenic activity of individual fractions established in this investigation.

Inasmuch as the nucleoprotein fraction is isolated from cellular cytoplasm, undoubtedly, it contains also nucleoproteins of cellular organoids, and therefore the observation of antigenic activity in the fraction of mitochondria – microsome may be regarded as antigenic activity of one part of the antigenic complex of the common nucleoprotein fraction.

The question of whether or not globulin is present in the nucleoprotein fraction and what its role is in the antigenic reaction brought about by nucleoprotein is awaiting further study and is the subject of the next communication.

The presence of specific tumor antigen in cellular organoids and in hyaloplasm proteins indicates the wide distribution of this antigen through various parts of the tumor cell.

On further investigation it is necessary to study the presence of specific tumor antigen in fractions from other cellular organoids (nuclei) as well as other fractions of hylaoplasm proteins (globulins isolated at 66% saturation with ammonium sulfate, albumins) and compare the antigenic properties of various fractions.

The experiments described indicate that mitochondria – microsomes and globulin fractions, isolated from hepatoma tissues and from breast concer of inbred mice, contain a specific tumor antigen which manifests itself in anaphylactic reaction with desensitization in guinea pigs. This specific antigen is also detectable in the fraction isolated from both tumors by the method of methanol precipitation. The detection of the specific tumor antigen in cellular organoids and in hyaloplasm proteins indicates the wide distribution of this antigen in various parts of the tumor cell.

SUMMARY

The presence of specific antigens was studied in certain fractions of mice tumors, which were induced and transplanted on purebred animals and, thus, had no isogenic variations.

It was established by anaphylactic reaction with desensitization in guinea pigs that there is a specific antigen present in the fractions of mitochondria, microsome, globulin and methanol precipitate of the hepatoma tissue and breast cancer in mice C_3HA. This antigen differed from the antigens which were present in the same fractions obtained from the normal liver or a mixture of organs of healthy mice.

The presence of specific tumor antigens in the cellular organoids, as well as in the proteins of the hyaloplasm shows the widespread distribution of this antigen in various parts of the tumor cell.

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IMMUNOCHEMICAL PROPERTIES OF VARIOUS FRACTIONS OF CANCEROUS AND NORMAL HUMAN TISSUES

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In our earlier investigations of quantitative serological reactions, we studied the immunochemical properties of saline and trichloroacetic extracts of cancerous and normal human tissues as well as their fractions: proteins, polysaccharides, lipids and residual antigen. In a previous study [1, 2] it was shown that the protein fraction and the residual antigen are serologically inactive, whereas the polysaccharide and lipid fractions possess activity. Moreover, the polysaccharide fraction of the "complete" antigen possesses the same serological activity as the original "complete" antigen; the polysaccharide fraction of the saline extract is 10-200 times more active than the original extract. "Complete" antigen is 10-90 times more active than the saline extract of the same tissues.

Thus, extraction with trichloracetic acid and acid hydrolysis of tissues lead, as it were, to concentration of serologically active substances.

In this study we investigated the antigenic properties of these serologically active fractions of cancerous and normal tissues of the human stomach.

EXPERIMENTAL METHOD

Seven rabbits were immunized with complete antigen obtained from human tissues by extraction with trichloracetic acid, according to Boiven's method; four of these were immunized with complete antigen obtained from cancerous stomach tissue, and 3 – with complete antigen of normal tissue. A total of four injections of antigen, one every other day, was given in the ear vein in amounts depending on its protein content. The amount of protein given was increased with each consecutive injection by 25-30%. A total of 3 mg of protein was administered. On the 7th and 11th day after the final injection blood was withdrawn for tests. The antibody titer was 1:10, 1:20. A month later these rabbits were reimmunized. Antigen was injected intravenously every third day for a total of 5 injections. The total amount of protein injected during the immunization was 16.4 mg. Blood was withdrawn on the 8th and 11th days after the final injection. The antibody titer was 1:80, 1:160.

Polysaccharide fractions representing polysaccharide – protein mixture were obtained with Morgan's method [3] by oxygen hydrolysis of saline extracts of cancerous and normal stomach tissues. Eight rabbits were immunized: four with the polysaccharide fraction of cancerous stomach tissue and four – of normal tissue. The plan of immunization and the quantity of protein administered were the same as in immunization with complete antigen.

Polysaccharide fractions were found less antigenic as compared to trichloroacetic extracts: their antisera had an antibody titer of 1:20 and 1:40. Thus, both complete antigen and polysaccharide fraction of cancerous and normal tissues of the human stomach possess antigenic properties. The lower antigenicity of the polysaccharide fractions can probably be explained by the intense alteration, in the course of obtaining the fraction

TABLE 1

Determination of Cancerous Specificity in Complete Antigen, Polysaccharide and Lipid Fractions of Cancerous Tissue of the Human Stomach

Animal No.	Sensitization		Demonstration of similarity among antigens						Demonstration of dissimilarity among antigens					
			testing for completeness of desensitization			resolving injection			testing for completeness of desensitization			resolving injection		
	antigen	dose of protein in mg	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg (for lipids – mg dry wt	reaction	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction
1	Saline extract of cancerous tissue of the human stomach	2	Saline extract of normal tissue of human stomach	4	—	Trichloracetic acid extract of cancerous tissue of the human stomach, complete antigen	4	++(+)	Trichloracetic extract of cancerous tissue of the human stomach	4,5	—	Saline extract of cancerous tissue of the human stomach	4	+
2		2		4	—		4	++		4,5	—		4	±
3		2		4	—		4	+(+)		4,5	—		4	++
4		2		4	—		3,5	+++		4,5	—		4,5	+
5 Control		.		.	.		4	—		.	.		.	.
1		2		4	—	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	4	+++	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	4,5	—		4	—
2		2		4	—		4	++		4,5	—		4	+
3		2		4	—		4	+++		4,5	—		4	+
4		2		4	±		4	+++		4,5	—		4	±
5 Control		.		.	.		4	—		.	.		.	.
1		2		4	—	Lipid fraction of saline extract of cancerous tissue of the human stomach	30	++	Lipid fraction of saline extract of cancerous tissue of the human stomach	30	—		4	±
2		2		4	—		30	+(+)		30	—		4	+
3		2		4	—		30	++		30	—		4	+
4		2		4	—		30	++		30	—		4	+
5 Control		.		.	.		30	—		.	.		.	±
Control		.		4	—		.	.		.	.		.	
Control		.		.	.		.	.		.	.		4	—

Remarks: The same and identical cancerous human tissue was used as material in the preparation of sensitizing and resolving antigens.

Designations: — absence of reaction; + momentary scratching of the nose; ++ strong scratching, ruffling of fur, sneezing, dyspnea, cough; +++ same, but more marked, elimination of urine and feces; ++++ convulsive jumping, convulsions usually terminating in death of the animal; dot — no injection; ± doubtful reaction.

The designations of desensitizing injections are omitted.

TABLE 2

Determination of Cancerous Specificity in Polysaccharide and Lipid Fractions of Cancerous Tissue of the Human Stomach

Animal No.	Sensitization		Second desensitization			Testing completeness of desensitization			Resolving injection		
	antigen	dose of protein in mg (for lipids mg of dry weight	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction
1	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	2	Saline extract of normal tissue of the human stomach	1,5	+++	Saline extract of normal tissue of the human stomach	4	—	Saline extract of cancerous tissue of the human stomach	4	++
2		2		1,5	++		4	±		4	+++
3		2		1,5	+		4	—		4	++
4		2		1,5	++		4	—		4	++
1	Lipid fraction of saline extract of cancerous tissue of the human stomach	30		1,5	++		4	—		4	+
2		30		1,5	+		4	—		4	++
3		30		1,5	++		4	—		4	++
4		30		1,5	±		4	—		4	±
Control (2 aminals)		.		.	.		4	—		.	.
		.		.	.		.	.		4	—

Remarks: The same and identical cancerous human tissue was used as material in the preparation of sensitizing and resolving antigens.
Designations: — absence of reaction; + momentary scratching of nose; ++ strong scratching, ruffling of fur, sneezing, dyspnea, cough; +++ same, but more marked elimination of urine and feces; ++++ convulsive jumping, convulsions usually terminating in death of the animal; dot — no injection; ± doubtful reaction. The designations of desensitizing injections are omitted.

(hydrolysis, treatment with ether, etc.), in their protein component which is mainly responsible for the antigenicity of the preparation.

In a later study we investigated the presence of cancerous specificity in the complete antigen and the polysaccharide and lipid fractions of the saline extract of cancerous tissue of the human stomach by making use of the anaphylactic reaction with desensitization.

Twelve guinea pigs weighing 280-400 g were sensitized by hypodermic injections of saline extracts of cancerous stomach tissue. The amount of antigen administered was based on its protein content, which was determined by Conway's method. Lipids were weighed when dry. Desensitization was produced 21 days later with a saline extract of normal stomach. On the day before, all pigs were desensitized with an intraperitoneal injection of antigen from normal stomach in the amount equal to three times the sensitizing dose. On the day of the experiment desensitization was accompanied by intracardiac injection every $1\frac{1}{2}$ - 2 hours until complete absence of reaction to the administration of antigen. For the resolving injection the animals were divided into 3 groups of 4 pigs each. The pigs in group I were given complete antigen from cancerous stomach tissue as the resolving antigen; the pigs in group II – polysaccharide fraction; the pigs in group III – lipid fraction.

The results of the experiments are given in Table 1.

As shown in Table 1, the pigs responded with 2-3 plus reaction in all instances. On the basis of data obtained, one may think that complete antigen, polysaccharide and lipid fractions of cancerous tissue participate in determining cancerous specificity. In order to determine whether or not all cancerous specificity is removed by lipid, polysaccharide and "complete" antigen, separately administered, the experimental animal was given an intracardiac injection of saline extract of cancerous stomach tissue, after examining the completeness of desensitization in relation to these fractions. The pigs responded with a weak reaction. Apparently the saline extracts contain substances other than lipid, polysaccharide and complete antigen responsible for cancerous specificity.

In order to finally settle the question of participation of the serologically active polysaccharide and lipid fractions in the production of cancerous specificity, use was made of anaphylactic reaction with desensitization under somewhat different circumstances so that one could at the same time evaluate the sensitizing capacity of these fractions.

Guinea pigs weighing 280-400 g were sensitized with polysaccharide and lipid fractions of cancerous tissue of human stomach. On the 21st day the animals were found to be sensitive to the cancerous as well as normal component of the tumor; on their desensitization by the introduction of saline extracts of normal tissue of the human stomach, the pigs responded with an anaphylactic reaction.

After desensitization, a saline extract of cancerous stomach tissue was used as a resolving antigen. The results of the experiments are shown in Table 2.

As shown in Table 2, upon sensitization of guinea pigs with polysaccharide and lipid fractions of cancerous tissue and after desensitization to normal tissues, the animals respond to the introduction of saline extract of cancerous stomach tissue with a marked anaphylactic reaction.

Thus, the lipid and polysaccharide fractions investigated not only participate in the production of cancerous specificity, but are also capable of sensitizing animals to cancerous and normal human tissue. Antigenicity of the polysaccharide – its capacity to sensitize pigs and to produce antibodies in rabbits – is easily explained. The polysaccharide fraction obtained with Morgan's method contains 3-4% of nitrogen, apparently, due to protein admixture. This cannot be said of the lipid fraction. When 20 mg of lipid fraction was burned we were unable to detect nitrogen with Conway's microtechnic. Despite this, the fraction sensitizes pigs to cancerous human tissues. Apparently, this occurs due to microadmixture of protein which is undetectable by chemical methods but is detectable by such a highly sensitive reaction as the anaphylactic reaction with desensitization, or because the given fraction actually does not contain protein but when introduced into the organism combines with its proteins and becomes antigenic.

In order to become convinced that the antigenicity of the polysaccharide fraction as well as the complete antigen depends on the presence of protein, we studied the amino acid content of these fractions by the method of distributive chromatography on paper. The solvent used was a mixture consisting of butanol, acetic acid,

and water (40 : 10 : 50). As a result of chromatographic examination 19 amino acids were identified in the preparations: cystine, lysine, histidine, arginine, aspartic acid, serine, glycine, hydroxyproline, glutamic acid, threonine, α-alanine, trolin,* tyrosine, aminobutyric acid, methionine, valine, phenylaline, isoleucine, lucine. The preparations investigated did not differ so far as qualitative content of amino acids was concerned, but only by the intensity of color spots was it possible to conclude that various preparations contained different amounts of amino acids.

The presence of such a large variety of amino acids in the fractions studied testifies to the fact that protein is one of their components which, apparently, determines their antigenic properties.

Therefore, this project demonstrates that the complete antigen and the polysaccharide fraction of the saline extract of cancerous human tissues possess antigenicity.

Thus, anaphylactic reaction with desensitization demonstrates the presence of cancerous specificity in lipid and polysaccharide fractions and in the complete antigen. It is possible that specificity of cancerous human tissues is determined by a combination of substances – polysaccharides and lipids along with proteins.

For consultation in the matter of chromatography technic we wish to thank A. N. Bitkova.

SUMMARY

Complete antigen obtained according to Boiven's method, and polysaccharide fractions of saline extracts of cancerous and normal tissues of the human stomach possess antigenic properties. The presence of a protein component in these fractions was established by the method of chromatography on paper. Existence of cancerous specificity in lipid and polysaccharide fractions and in complete antigen of human tumor tissue was demonstrated by the reaction of anaphylaxis with desensitization.

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THE EFFECT OF DIMEDROL* ON THE DEVELOPMENT OF SHWARTZMAN'S PHENOMENON

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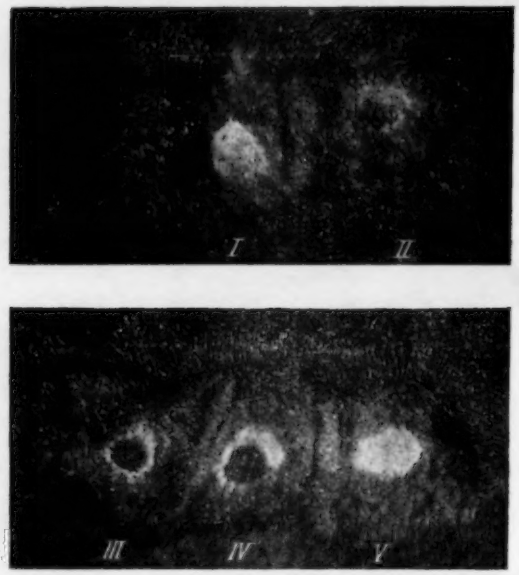
Dimedrol – one of the best domestic histamine preparations – was found to be quite effective a remedy in the treatment of allergic diseases of various origins. However, there are no exact indications for its application, which is probably due to scarcity of experimental data on the mechanism of its action. For this reason we undertook the problem of studying the effect of dimedrol on the development of Shwartzman's phenomenon.



Development of Shwartzman's phenomenon in rabbits by experimental administration of dimedrol. From left to right: skin area I) negative Shwartzman's phenomenon (no skin changes); skin areas II) positive Shwartzman's phenomenon bluish hemorrhagic spots); skin area III) positive Shwartzman's phenomenon; skin area IV) positive Shwartzman's phenomenon; skin area V) no skin changes.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits. The phenomenon was produced by the method described by Shwartzman. Dimedrol was administered in 1 ml doses of a solution of 1:10,000 (solutions of concentration greater than 1:1,000 were found to be toxic and produced skin necrosis when injected intracutaneously). In control experiments pure dimedrol or physiological solution was used. In other experiments ascorbic acid (10-50 mg) was used; according to the literature, its intracutaneous injection averts the development of Shwartzman's phenomenon [2].

Five skin areas were prepared on the lateral surface of the body of rabbits weighing not less than 1,500 g with the use of a depilator; into each one of these areas 0.5 ml of a filtrate of B. coli was injected.

In the first variant of the experiments dimedrol was injected intracutaneously simultaneously with the preparatory injection.

Into skin area I were injected a filtrate of B. coli and dimedrol; into area II – a filtrate of B. coli and physiological solution; into area III – a filtrate of B. coli only; into area IV – a filtrate of B. coli and 10 mg of ascorbic acid (2 hours before the resolving injection); into area V – dimedrol only. Twenty-four hours later the resolving injection was given (1 ml of filtrate of B. coli intravenously).

In the second variant of the experiments dimedrol was injected into those skin areas into which previously

*Diphenhydramine hydrochloride.

a filtrate of B. coli and the resolving injection were simultaneously introduced, i.e., 24 hours after the preparatory injection. The order of the intracutaneous injections of preparations was the same as in the preceding experiment. The dose of ascorbic acid was 30 mg.

In the third variant of the experiments dimedrol was injected intracutaneously one hour after the resolving injection, i.e., 25 hours after the preparatory injection. The order of the intracutaneous injections of preparations was the same as in the preceding experiment. The dose of ascorbic acid was 50 mg.

In all of the three variants of the experiments the results were identical and consisted of the following (see figure): skin area I – negative reaction (no skin changes); area II – positive reaction (bluish hemorrhagic spots); area III – positive reaction; area IV – positive reaction; area V – negative reaction (no skin change).

Thus in all three variants of the experiments dimedrol averted the development of Shwartzman's phenomenon.

Shwartzman [4] demonstrated that following appropriate preparation the phenomenon can be observed in the viscera as well: in the lungs and kidneys. This fact was confirmed by Gratsiia.

We set up a series of experiments with the purpose of determining whether or not Shwartzman's phenomenon could be averted in the viscera as well by administration of dimedrol. For this purpose 8 mg of dimedrol per 1 kg weight of rabbit was administered intravenously and intramuscularly simultaneously with the preparatory injection and also on the second and third days of the experiment.

In not one of the variants of the experiments did we observe that the development of Shwartzman's phenomenon was averted in the viscera.

Thus dimedrol averts the development of Shwartzman's phenomenon in rabbits upon its introduction in skin areas prepared with filtrate simultaneously with the preparatory injection, simultaneously with the resolving intravenous injection of the filtrate, and one hour after the resolving injection of the filtrate.

At the same time intravenous and intramuscular administration of dimedrol even in massive doses does not avert the development of Shwartzman's phenomenon.

When ascorbic acid was introduced into the preparatory areas it too did not avert the development of Shwartzman's phenomenon in a single variant of our experiments.

SUMMARY

It was established that dimedrol averted the development of Shwartzman's phenomenon in rabbits when administered intracutaneously as follows: 1) simultaneously with the preparatory dose of filtrate of B. coli, 2) simultaneously with the resolving dose of filtrate of B. coli and 3) one hour after the administration of the resolving dose of filtrate of B. coli. Dimedrol did not avert the phenomenon in viscera.

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ONCOLOGY

EXPERIMENTS ON HETEROTRANSPLANTATION OF HUMAN TUMORS INTO CORTISONE-TREATED RATS AND HAMSTERS

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Although attempts at transplantation of human tumors were begun a very long time ago, it is only very recently that repeated passage of such tumors through animal hosts has been successful. It is obvious that the availability of human tumor strains which have been transplanted from animal to animal for prolonged periods of time opens up wide research possibilities for workers in various fields of experimental oncology [2, 5]. It is only during the past few years that certain workers [6-8] succeeded in obtaining a few strains of human tumors which could be repeatedly passaged through animals, using cortisone to inhibit immune reaction of the host. Considerable difficulties were encountered in maintaining these strains. Thus H. Toolan [8] transplanted over a thousand human tumors into rats and hamsters, and only 5 strains emerged which could be passed repeatedly from animal to animal.

Such strains have not yet been reported in the Soviet literature. Those authors who performed heterotransplantation of human tumors into chick embryos and other animals were able to maintain the transplants for not more than a few passages [1, 3, 4].

The present paper describes the results of transplantation of 90 human tumors into rats and hamsters.

EXPERIMENTAL METHODS

Tumor tissues were obtained from the Clinical Division of the Institute of Experimental Pathology and Therapy of Cancer, AMN SSSR, and were inoculated within 30-40 minutes into the cheek pouches of golden hamsters, aged 1-2 months, or subcutaneously into 3- to 4-week-old rats. The tissue was first treated with penicillin (10,000-15,000 units per ml of physiological saline) for 10 minutes, and was then cut up into fragments with the aid of a pair of scissors, in a Petri dish. The hamsters were anesthetized with ether, the cheek pouches were turned inside out and exteriorized, a fold of mucosa was raised with the aid of a pair of forceps, cleaned with a sterile swab, and the tumor tissue suspension was injected submucosally. Cortisone was given subcutaneously. The cheek pouches were everted every 15-20 days and examined for growth of the transplant. The rats were inoculated with tumor tissue subcutaneously, in the usual way. Injections of 5 or 2.5 mg of cortisone were given to the rats and hamsters immediately after inoculation, and twice weekly thereafter.

EXPERIMENTAL RESULTS

The human tumor material transplanted into rats included the following: 31 sarcomas from various sites, 8 melanomas, 11 gastric carcinomas, 3 ovarian carcinomas, 2 mammary carcinomas, 3 seminomas, 3 laryngeal carcinomas, and 1 lung carcinoma. Slight growth was found in the first generation (during the first 10-12 days

after inoculation) for 10 sarcomas, 3 melanomas, 2 gastric carcinomas, 1 lung carcinoma, and 2 laryngeal carcinomas; in the second generation we found growth of 4 sarcomas, 2 gastric carcinomas, and 1 melanoma. Growth was, however, insignificant in all cases, and in no case could we achieve prolonged serial transmission of a tumor.

Human tumor tissues transplanted into hamsters included 13 sarcomas, 2 gastric carcinomas, 4 melanomas, 3 mammary carcinomas, 2 lung carcinomas, and 3 laryngeal carcinomas. As noted above, they were injected twice weekly with doses of 5 or 2.5 mg of cortisone.

In the first group (16 tumors, cortisone dosage level 5 mg) the hamsters died as a result of the toxic action of cortisone during the first 10 days after inoculation; in no case was any significant growth of tumor tissue observed. The dosage of cortisone was then reduced to 2.5 mg, and this resulted in a much longer survival time of the hamsters. We implanted 25 tumors at this dosage level. Slight growth of 1 of 2 melanomas and of 1 of 2 lung carcinomas was observed in the first generation; 14 carcinomas taken from different sites (including mammary, lung, and others) gave no marked growth.

The best results in this series were obtained with sarcomas. Considerable growth of 4 of 8 human sarcomas implanted into the cheek pouches was seen; one of them (spindle-cell uterine sarcoma) grew vigorously during the first 3 serial passages, but failed to grow thereafter. Two other tumors (chondrosarcoma and thymoma) survived to the 4th and 3rd generations, respectively; further passage of these tumors is in progress. One of the tumors has now been maintained by serial passage through hamsters for over 15 months, and is now in its 14th generation.

We shall give a more detailed description of the properties of this tumor, which we have designated as HT-67, as it was the 67th human tumor taken for our transplantation experiments.

A tumor, diagnosed clinically as a sarcoma of the abdominal wall, was removed surgically from the patient Sh., on May 23, 1956. Histological examination showed that it was an angiosarcoma which had infiltrated the muscle and connective tissue. The patient died 23 days after the operation. At autopsy a recurrence of the angiosarcoma was found in the anterior abdominal wall, with invasion into the abdominal wall and dissemination into the visceral peritoneum of the large and small intestines, and with metastatic deposits in the left lung.

The tumor, which was a soft, milk-white mass, was implanted immediately after removal into the cheek pouches of 5 hamsters and subcutaneously into 4 rats; all the animals were given cortisone. After a month the rats had tumors of up to 1 cm diameter; these tumors were transplanted into a fresh group of rats, but they failed to grow. Of the 5 hamsters, 3 died during 15 days after implantation, for a variety of reasons. Growth of the implant was seen in one of the survivors at the end of a month. On the 43rd day after implantation a tumor of about 1 cm diameter was present, and this was transplanted into 3 fresh hamsters; the implants all showed growth by the end of a month, and the tumor was passed serially through a succession of hamsters. The biological properties of the tumors (inoculability, rate of growth, etc.) varied fairly widely from passage to passage, in an irregular manner.

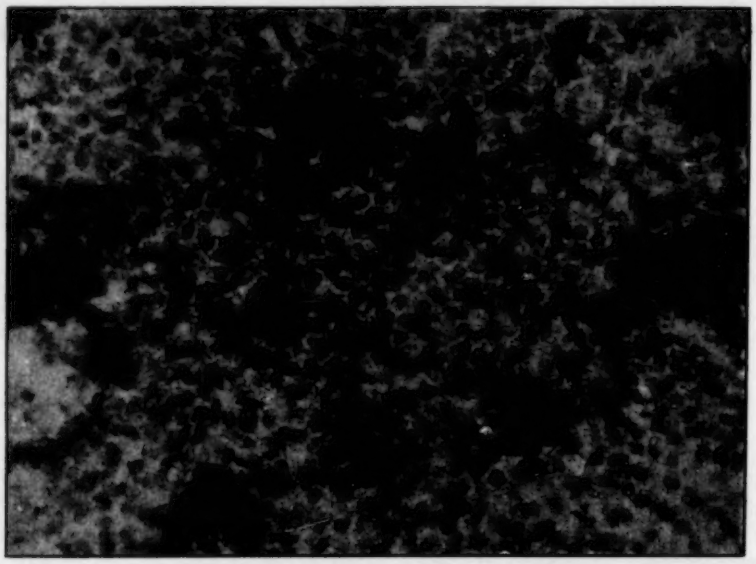
During the first 11 generations we found that 68.4% of the transplants gave rise to tumors (calculated for each cheek pouch separately). Tumors were usually taken for transplantation when they had achieved a diameter of 1-1.5 cm. In some generations this was achieved within 11-16 days, while in other animals there was a latent period of up to $2\frac{1}{2}$-3 months. The number of animals at our disposal was limited, and wastage due to various complications resulting from the toxic effects of cortisone was fairly considerable; for these reasons we have not yet been able to accumulate sufficient experimental data to justify any firm statements as to the final fate of the tumor-bearing hamsters. Individual observations showed, however, that growth of HT-67 tumor may proceed for a long time (up to 4-5 months) in the cheek pouches, and that the diameter of the tumors may be 2-3 cm or more.

We have hardly ever observed resorption of the tumors. In one case only did we find that a tenth generation tumor, which had reached a diameter of 1 cm at the end of the 3rd month of growth, underwent total resorption during the course of a few days.

Attempts at transplanting HT-67 tumor which had been passed through a series of hamsters into cortisone-treated rats were unsuccessful; tumor growth was not observed in any of the inoculated rats.

The histological structure of angiosarcoma HT-67 deviated very little from that of the original tumor

during its passages. The transplants grown in hamsters consisted of large, atypical connective tissue cells with large oval or round, faintly staining nuclei (see figure); the arrangement of these cells was in most cases a disorderly one, and only in certain parts of the tumor could we see any tendency toward formation of parallel rows of cells. In many, although not in all, of the transplanted tumors there were numerous sinusoidal dilatations of the capillaries, which were lined with atypical tumor cells; the lumina of these capillaries were filled with erythrocytes.



Histological structure of a human angiosarcoma (Strain HT-67) taken from its 12th passage through hamsters. Numerous sinusoidally dilated capillaries are to be seen. Photomicrograph. Magnification 300 ×.

Our experiments have revealed certain factors of importance in work on the heterotransplantation of tumors into cortisone-treated animals. Among such factors are, above all, the species of animal, and the site of implantation ; transplantation into the cheek pouches of hamsters gave a higher proportion of takes than did subcutaneous inoculation into rats. Moreover, even HT-67 tumor, which grew actively for a long time in the cheek pouches of hamsters, failed to grow when transplanted into rats. We cannot suggest any explanation of this difference, but it is evident that hamsters are more suitable than rats for the heterotransplantation of human tumors. The histological form of the neoplasm is also of importance; sarcomas appear to give active growth more frequently than do tumors of epithelial origin. The HT-67 tumor, which has been passed serially through hamsters, may be used for various purposes. As was noted above, this angiosarcoma retained its initial structure through numerous passages. The full elucidation of the effects of prolonged heterotransplantation on the properties of this human tumor must, however, await the results of special investigations, involving the application of cytological (study of chromosome complement), immunological, and tissue culture methods.

SUMMARY

Human malignant tumor tissues, taken from 90 patients, and including carcinomas, melanomas, and sarcomas from various sites, have been implanted into the cheek pouches of golden hamsters, and subcutaneously into rats, all cortisone-treated. Growth of the implants was found in a number of cases, but continued growth over a number of serial passages was achieved only with one tumor (an angiosarcoma) in hamsters. This tumor has been passed serially through 14 generations for over 15 months, over which time it has retained its original histological structure. It could not be transplanted from hamsters to rats.

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EXPERIMENTAL BIOLOGY

PRELIMINARY RESULTS OF A STUDY OF BACTERIAL PROTOPLASTS

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The cell wall is an important component of the bacterial cell, its basic function being to shield the cell contents from unfavorable environmental factors, as well as to maintain the characteristic shape of the cell Addition of lysozyme to a suspension of cells which are sensitive to this specific enzyme, acting on polysaccharides, leads to breakdown of the cell wall, followed by release of the cell contents, which diffuse into the medium [1, 11, 12]. It was first shown by Weibull [15, 16] that in the presence of certain "stabilizers" (various substances, such a sodium chloride or sucrose, at definite concentrations) the cell contents do not diffuse into the medium after the cell wall has been broken down by the lysozyme, but form a spherical body, which he called a protoplast. Later work showed that protoplasts are capable of performing certain vital functions: they respire, they can assimilate labelled amino acids, and they can synthesize protein, as a result of which their nitrogen content rises, as well as their weight [2, 3, 6, 7, 13], bacteriophage can multiply within them [5, 9, 12] and spores can develop within them [10]. Microscopic and electron microscope examination of protoplasts has shown the presence of mitotic figures, being evidence of their multiplication [8]. All these observations support the view that protoplasts are not merely lumps of surviving protoplasm, but that they represent a definite form of extracellular life. The question naturally arises as to whether regeneration of protoplasts, to produce secondary bacterial cultures, is possible.

The opinion has recently been expressed [14] that protoplasts are identical with L-forms. This view is supported by the observation that penicillin acts on Gram-negative bacteria (this is the usual procedure for production of L-forms) in the presence of sucrose, as a stabilizer, to give protoplasts, the morphology and mechanism of formation of which resemble those of L-forms [4].

The objects of our research were to produce protoplasts, to study their morphology and functional properties, and then to attempt to achieve regeneration of the protoplasts into secondary bacterial cultures.

EXPERIMENTAL METHODS

In our experiments we used washings of 18-20-hour cultures of B. megatherium grown on a solid synthetic nutrient medium, as well as 18-20-hour cultures in 2% peptone water to which a lysozyme preparation had been added (white of fresh hen's egg) in various proportions, from 1:100 to 1:1.

The microbes were washed from the solid nutrient cultures by means of M/30 phosphate buffer (pH 7.6) containing 10% of sucrose and lysozyme (experiment) or of buffer and lysozyme without sucrose (control 1). The second control (2) involved washing off the organisms with buffer containing sucrose, but not lysozyme. With 2% peptone water nutrient medium the experimental systems contained 10% of sucrose, control 1 was 2% peptone water with lysozyme but without sucrose, and control 2 was 2% peptone water with 10% of sucrose, but without lysozyme.

The experimental and the control suspensions were incubated at 37° for various times, for 15 minutes to

3 hours, and the organisms were then separated from the lysozyme by centrifuging (6000 rpm for 15 minutes). The residues so obtained were resuspended in the appropriate solutions not containing lysozyme, and the suspensions were examined in hanging drop and in stained preparations (fixed with 4% formalin for a number of days in a refrigerator). The organisms were stained according to Gram, and with Loeffler's methylene blue.

Respiration was measured by Warburg's method. The results were expressed as cubic millimeters of oxygen absorbed per ml of suspension in 30 minutes.

The proportions of unchanged cells to protoplasts were checked by inoculation on meat-peptone agar with and without sucrose.

EXPERIMENTAL RESULTS

Our comparative study of formation of protoplasts from B. megatherium M. lysodeicticus, and B. mesentericus cells showed that lysozyme caused total lysis of M. lysodeicticus cells, without formation of protoplasts. B. mesentericus was relatively resistant to lysozyme, only sporadic protoplasts being formed, while most of the cells remained unaffected. B. megatherium gave large numbers of protoplasts, with sporadic unaffected cells.

Microscopic Examination of Fixed Preparations

Smears from the experimental systems: intensely stained Gram-negative, homogeneous, spherical bodies, diameter 3-6 μ; sporadic bacillary forms of B. megatherium.

Control 1 (suspensions not containing sucrose): single bacillary forms of B. megatherium.

Control 2 (suspensions not treated with lysozyme): large numbers of unchanged bacillary forms of B. megatherium.

No formations resembling protoplasts could be seen in either of the controls.

Attempts at separating protoplasts from intact cells by centrifugation (3000 rpm for 15 minutes) were unsuccessful, as the larger protoplasts moved together with the larger cells, leaving the smaller protoplasts together with the smaller cells in the supernate.

Development of cells from protoplasts plated on meat-peptone agar containing sucrose was not observed. Growth of lysozyme-resistant cells took place, as in the control systems.

Comparison of respiration of protoplast suspensions with that of the initial culture gave no evidence of respiration of protoplasts.

Since we were unable to obtain suspensions of protoplasts reasonably free of unaffected cells we proceeded to the production of protoplasts from Gram-negative bacteria by treatment with penicillin.

Preparation of Protoplasts by the Action of Penicillin on Gram-Negative Bacteria (B. Coli 844; Salmonella Gallinarum 7979; Salmonella Gallinarum 398, Proteus X_{19})

Lederberg [4] showed in 1956 that regeneration of bacteria from their protoplasts was possible. His experimental material consisted of B. coli cultures. He used penicillin for breaking down the cell wall, with sucrose as the stabilizer. Lederberg followed the process of gradual formation of protoplasts, and of the subsequent transformation into cells in a penicillin-free medium.

EXPERIMENTAL METHODS

We used 18-20-hour cultures of Salmonella gallinarum in meat-peptone broth, and also 3-4-hour cultures for some experiments. The cultures were either not aerated, or were aerated by means of a magnetic stirrer. After incubation, 1 ml of the cultures was added to 10 ml of meat-peptone broth containing 10% sucrose, 0.2% of magnesium sulfate, and varying amounts of penicillin (from 100 to 2000 units/ml).

Control 1: the same, without sucrose; Control 2: the same, without penicillin.

The cultures were incubated for 6 hours at 37°, with or without aeration.

Samples of the cultures were plated out at hourly intervals on meat-peptone agar containing 10% sucrose, or without sucrose, and preparations were taken for microscopic examination (see techniques for examination of Gram-positive bacteria).

The optimum conditions for production of protoplasts were as follows: 1) concentration of microbial bodies; 1 ml of culture to 10 ml of meat-peptone broth; 2) final concentration of penicillin 1000 units/ml; 3) incubation conditions, 3 hours without aeration.

EXPERIMENTAL RESULTS

Formalin-fixed preparations for cultures containing penicillin and sucrose showed the presence of homogeneous spherical bodies 2-5 μ in diameter, giving fairly intense Gram-negative staining. Groups of 2, 4, or 6 protoplasts, diameter 1-2 μ, could be seen (Figs. 1, 2).

The controls without sucrose showed single unchanged Salmonella gallinarum bacilli, and clumps of amorphous material (evidently lysed cell debris). Large numbers of Salmonella gallinarum bacilli were seen in the control cultures without penicillin.

Formations resembling protoplasts were not found.

Tables 1 and 2 present the results of inoculating the experimental and the control cultures on meat-peptone agar containing sucrose and meat-peptone-agar not containing sucrose.

TABLE 1

Growth of Salmonella gallinarum 7979 on Meat-Peptone Agar With and Without Sucrose, after Different Durations of Exposure to Penicillin

Initial culture	Nutrient medium	Duration of action of penicillin		
		1 hr	2 hr	3 hr
Salmonella gallinarum in meat-peptone broth containing sucrose and 1000 units/ml of penicillin	Meat-peptone agar containing sucrose	+++	++	+
	Meat-peptone agar	++	++	−
Salmonella gallinarum in meat-peptone broth without sucrose, containing 1000 units/ml of penicillin	Meat-peptone agar containing sucrose	++	++	−
	Meat-peptone agar	+++	++	−
Salmonella gallinarum in meat-peptone broth containing sucrose	Meat-peptone agar containing sucrose	Over 50 colonies		
	Meat-peptone agar			

Explanation of symbols: + single colonies; ++ over 15 colonies; +++ over 30 colonies; − no growth.

Examination of stained smears taken from colonies grown on meat-peptone agar containing 10% sucrose (fixed in 4% formalin) supported the view that these colonies consisted of cells which had developed from protoplasts; the cells were polymorphous, and the preparations contained sporadic protoplasts 2-3 μ in diameter, as well as smaller ones (0.5-1 μ) distributed in groups of three (Fig. 3).

Comparative studies were made of the respiration of the cells and of their protoplasts (Tables 3 and 4).

The respiration of the cells always considerably exceeded that of the protoplasts. Very low respiration, much less than in suspensions of protoplasts containing sucrose, was found in the control suspensions containing penicillin but not sucrose (due evidently to cells on which penicillin had not acted). Only in a few of the experiments did penicillin totally abolish respiration.

We have thus confirmed that breakdown of the cell wall of bacteria by specific agents (lysozyme, penicillin) in the presence of stabilizer (sucrose) is associated with the formation of spherical bodies, analogous to Weibull's protoplasts.

Fig. 1. Formation of protoplasts from Salmonella gallinarum cells.

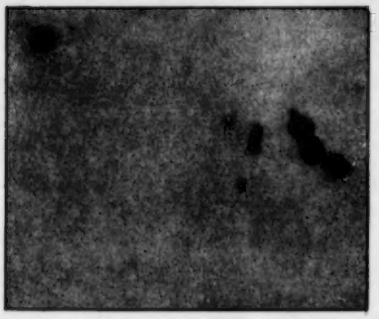

Fig. 2. Division of Salmonella gallinarum protoplasts.

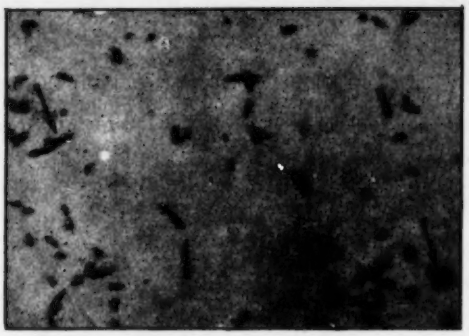

Fig. 3. Protoplasts in a smear from a colony of Salmonella gallinarum grown on meat-peptone agar containing 10% sucrose.

TABLE 2

Growth of Salmonella gallinarum 7979 and of Salmonella gallinarum 398 after Exposure to the Action of Penicillin for 3 Hours.

Nutrient medium	Initial culture					
	microbial culture in meat-peptone broth containing sucrose and 1000 units/ml of penicillin		microbial culture in meat-peptone broth containing sucrose and 1000 units/ml of penicillin		microbial culture containing 1000 units/ml of penicillin, without sucrose	
	strain of Salmonella gallinarum					
	7979	398	7979	398	7979	398
Meat-peptone agar containing sucrose	++	++	–	+	Over 50 colonies	
Meat-peptone agar	–	–	–	–		

Explanation of symbols: as in Table 1.

Mitotic figures were seen in fixed smears of B. megatherium and S. gallinarum protoplasts, showing that these may have the capability of development. Respiratory activity was also observed with S. gallinarum protoplasts; it was quite pronounced, although less than with suspensions of the intact organisms.

The best material for preparation of protoplasts was S. gallinarum subjected to the action of penicillin in the presence of 10% sucrose as stabilizer. In conclusion, we would like to thank Prof. G. P. Kalina for supervizing this research.

TABLE 3

Comparison of Respiration of Various Bacteria and of Their Protoplasts

Microbial species	Initial culture		
	microbial culture in meat-peptone broth containing sucrose	microbial culture in meat-peptone broth containing sucrose and 1000 units/ml of penicillin	microbial culture containing 1000 units/ml of penicillin, without sucrose
Salmonella gallinarum 7979	278	50	–
Salmonella gallinarum 398	248	37	9
B. coli 844	169	56	10
Proteus X_{19}	269	190	127

TABLE 4

Relation Between Respiration of Salmonella gallinarum 7979 Cells and Protoplasts and the Concentration of Penicillin (cubic millimeters of oxygen absorbed per ml of suspension in 30 minutes)

Initial culture	Concentration of penicillin					
	0 units/ml	2000 units/ml	1000 units/ml	500 units/ml	250 units/ml	100 units/ml
In meat-peptone broth containing sucrose	68	–	32	50	50	55
In meat-peptone broth	61	–	10	16	23	25

SUMMARY

Spherical bodies resembling Weibull's protoplasts are formed when certain bacteria (B. megatherium Salmonella gallinarum, B. mesentericus) are incubated with lysozyme or penicillin in the presence of 10% sucrose, as a stabilizer. The presence of mitotic figures in some of the protoplasts suggests that they are capable of development and regeneration.

The S. gallinarum protoplasts showed some respiratory activity, although much less than the intact cells. B. megatherium protoplasts did not respire.

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EFFECT OF PHYSIOLOGICAL CONDITION OF ASCARIDS ON THEIR SURVIVAL TIME IN AN ATMOSPHERE OF OXYGEN

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A number of authors have shown the value of oxygen in the treatment of ascaridosis [1, 3, and others]. N. P. Kravets found that ascarids died after an hour of exposure to oxygen, as compared with a survival time of up to 10 hours in air [2]. H. Laser [7] and A. I. Krotov [5] have shown that the metabolism of ascarids is to a certain extent an aerobic one. In the presence of excess of oxygen they are, however, unable to dispose of the hydrogen peroxide accumulating as a result of respiratory processes, owing to their low catalase activity. The accumulation of hydrogen peroxide appears to cause their death when they are exposed to an atmosphere of oxygen.

EXPERIMENTAL METHODS

We determined the catalase activity of swine ascarids by Bach's manganometric and gasometric methods (1937), and we studied their survival time in an oxygen atmosphere under various conditions. The catalase activity varied for each batch of ascarids received from the slaughter house, for which reason it was estimated as a separate control for each series of experiments. The catalase activity index was taken as being the number of milligrams of hydrogen peroxide decomposed in 1 hour by the catalase present in 1 ml of body-cavity fluid or ascarid muscle brei, at 20°.

Each determination was done in triplicate, and only those values which did not differ by more than 0.1% were taken as trustworthy. In all, we performed 450 manganometric and 35 gasometric estimations of catalase activity, of which 50% were controls. The student K. Kats performed some of the experiments.

The catalase activity index of ascarids varied for different batches, values of from 300 to 2050 being found. Catalase activity could not be found in certain batches of ascarids, and such batches were not taken for further experimentation.

EXPERIMENTAL RESULTS

It appeared from a large number of experiments that the catalase activity of the body-cavity fluid of ascarids did not differ significantly from that of the muscles, and we therefore based the bulk of our experiments involving determination of catalase activity on body-cavity fluid. The catalase activity did not appear to vary according to the age or the sex of the ascarids. The activity fell progressively with duration of storage of the worms, and for this reason we used freshly collected ascarids in all our experiments. When the ascarids were kept under anaerobic conditions we were unable to find any catalase activity; this appeared only a certain time after they were placed in salt solution in equilibrium with atmospheric air. The supply of nutrients significantly affected the catalase activity. When the ascarids were placed in saline solution without nutrients the catalase activity index had a mean value of 272, as compared with 850 for material from the same batch kept in saline containing glucose and proteins.

When the ascarids were subjected to periodic electrical stimulation for 30 minutes their catalase activity

fell to zero. It is known that the activity of ascarids is greatly diminished when they are immersed in chloral hydrate solution, whereas in piperazine or santonin solutions contraction of the body alternates with its relaxation, for the first few hours. Determination of catalase activity of ascarids while stimulated or inhibited by these substances gave the following results.

TABLE 1

Changes in Catalase Activity of Ascarids Kept Under Various Conditions

Duration of exposure of ascarids to the given solutions, in hours	Catalase activity index of the ascarids	
	piperazine (1:1000)	santonin (1:10,000)
0	1870	1250
1	240	0
2	0	0
3	0	0
20	850	680

In an inhibited state, caused by immersion in 0.1% chloral hydrate solution for 20 hours, the catalase activity index rose to values 72-83% above the normal. In a stimulated state, resulting from exposure to 0.01% santonin for 2-5 hours, the catalase activity index fell to zero in all cases. The same effect was found after exposure to 0.1% piperazine. The value of the catalase activity index again rose after prolonged exposure to santonin or piperazine (Table 1). This may be ascribed to the state of depression of activity which succeeds that of excitation of ascarids in such cases [6, 7].

Lowering of catalase activity was also found when the ascarids had been exposed to the action of a number of other anthelminthics, in particular, chenopodium oil, heptylresorcinol, and thymol.

Maintenance of the ascarids in methylene blue solution (1:10,000) for a day also resulted in the catalase activity index falling to zero, and it again rose after 2-3 days. It is known that, at certain concentrations, methylene blue causes intensification of aerobic metabolism of helminths, but at high concentrations it inhibits it. The survival time of ascarids exposed to an atmosphere of oxygen also depends on their physiological condition (Table 2). They were considered to be dead when they ceased to respond to electrical stimulation. Oxygen was passed at a uniform rate into the ascardiograph tube containing Locke's solution in which the ascarids were immersed. The state of the nematodes was registered on a kymograph [4].

TABLE 2

Relation Between Survival Time of Ascarids Exposed to the Action of Oxygen and Their Physiological Condition

Physiological condition of ascarids	Time in minutes elapsing before response to electrical stimulation is abolished
Normal – Locke's solution	45 – 55
Inhibition – 1% chloral hydrate	70 – 90
Excitation:	
electrical	15 – 30
santonin	30 – 40
piperazine	35 – 42

Our experiments permit the drawing of the following conclusions:

1. The intensity of aerobic respiration of ascarids varies according to their physiological condition, as was shown from determinations of their catalase activity and survival time when exposed to an atmosphere of oxygen.

2. Inhibition of motor activity of ascarids leads to a rise in catalase activity and in survival time in an atmosphere of oxygen. Conversely, catalase activity falls under conditions of stimulation, and the ascarids die sooner when exposed to oxygen.

3. The effectiveness of oxygen therapy for ascaridosis can be enhanced by preliminary administration (2-3 hours earlier) of small doses of piperazine or santonin.

SUMMARY

The ascaricidal effect of oxygen is related to accumulation of hydrogen peroxide formed as an aerobic metabolite under conditions of low catalase activity. Stimulation of motor activity of the ascarids by immersion in santonin or piperazine solutions or by galvanic current abolishes catalase activity, and thus shortens

survival time during exposure to oxygen. Conversely, abolition of motor activity by immersion in chloral hydrate solutions raises catalase activity, and prolongs survival time. Preliminary administration of small doses of santonin or piperazine should enhance the effectiveness of oxygen therapy for ascaridosis.

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PHOTOPERIODICITY AND THE FUNCTION OF THE HYPOPHYSIS AND THE ADRENAL CORTEX

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It was shown in an earlier paper [2] that a considerable fall (to 64% on the average) takes place in the eosinophil count of the peripheral blood of rats during the evening hours, as compared with the values found for blood samples taken in the morning. These rhythmic diurnal variations of eosinophil count were observed at all seasons of the year, but the highest counts were found at 2 p. m. in January, and at 6 p. m. in June. Disturbance of the normal day and night rhythm (24-hour illumination or darkness) leads to abolition of the eosinophil rhythm of peripheral blood. Inversion of normal photoperiodicity (illumination by night and darkness by day) leads to inversion of the eosinophil count rhythm. A correlation was thus found between the eosinophil count of the peripheral blood of rats and the environmental photoperiodicity. The diurnal eosinophil rhythm of rats was found to be disturbed within 8-13 days of bilateral adrenalectomy, pointing to the role of the adrenals in effecting the rhythm. The eosinophil rhythm is parallelled by a similar rhythm of urinary 17-ketosteroid excretion. Analogous effects were found by Brown and Dougherty in mice (1956). These authors reported rhythmic changes affecting not only the eosinophil count, but also the total leucocyte, lymphocyte, and neutrophil counts. The leucocyte count of blood taken from the tail vein during daylight hours was found by these authors to be 3-4 times higher than at night. Fourteen days after adrenalectomy mice no longer showed the diurnal rhythmic changes in eosinophil, lymphocyte, neutrophil, and total leucocyte counts.

Doe, Flink and Godsell (1956) examined the eosinophil count of healthy men, as well as the 17-hydroxycorticosteroid content of their blood and urine. They found that the 17-hydroxycorticosteroid content began to rise at 6 a.m. to a maximum at 9 a.m., falling thereafter to a minimum at 9 p. m. The eosinophil count began to fall at 6 a.m., reaching a minumum at 9 a.m., and then rising abruptly toward the evening.

Radnot, Wallner and Török (1956) found that the morning fall in eosinophil count of a series of patients took place only under normal conditions, but was abolished when the patients were kept in the dark.

Similar findings were reported by Migeon, Tyler, Mahoney, Florentin, Castle, Bliss and Samules (1956). These authors also found a considerable fall in the amplitude of the rhythmic fluctuations of corticosteroid hormones in blind persons.

There is, therefore, a diurnal rhythmic change in the eosinophil count of peripheral blood of humans, rats, and mice, as well as in the 17-keto- and 17-hydroxycorticosteroid contents of the blood and urine.

The object of our research was to find whether the photoperiodic fluctuations in eosinophil count of the blood of rats depend on the diurnal rhythm of the adrenocorticotrophic function of the hypophysis and of the endocrine activity of the adrenal cortex.

EXPERIMENTAL METHODS

We examined the content of adrenocorticotrophic hormone (ACTH) in the hypophyses of rats in the morning and the evening, i.e., at times corresponding with those of pronounced changes in eosinophil count.

The rats were killed by decapitation at 9 a.m. and in the evening (at 9 p.m. in June, at 6 p.m. in August and September, and at 4-2 p.m. in October to December). Extraction of ACTH from the hypophyses was effected by the method of Sydnor and Sayers (1954). The hypophyses were weighed and placed in 0.1 N hydrochloric acid (0.5 ml per hypophysis), and left in the refrigerator for a week. They were then ground up with glass powder, and the suspension was centrifuged; the supernate was then diluted to the desired volume with 0.01 N hydrochloric acid. The adrenocorticotrophic activity of the hypophyses was derived from the fall in ascorbic acid content of the adrenals of 4-7-day-old rats following intraperitoneal injection of the extracts, at a rate of 2.0-2.5 mg of hypophysis per rat (in 0.1-0.2 ml of solution). The rats were killed $1\frac{1}{2} - 2$ hours after the injection, and the adrenals were removed. Their ascorbic acid content was determined by the method of Roe and Kuether (1943). Each experimental group consisted of not less than 10 animals. One group served as a control, and was not injected, while two other groups were given injections of extracts of hypophyses of rats killed in the morning or evening.

TABLE 1

Effect of Extracts of Hypophyses of Rats Killed in the Morning or the Evening on the Ascorbic Acid Content of the Adrenals of Newborn Rats

Experimental conditions	Ascorbic acid content of the adrenals of baby rats (mg-%)		
	control group	given extracts of the hypophyses of rats killed in the	
		morning	evening
Normal illumination	247	173	154
	147	67	52
	185	70	41
	305	174	147
	—	93	31
	81	38	11
	183	126	89
24-hour illumination	195	128	133
24-hour darkness	—	88	80
	173	130	122

EXPERIMENTAL RESULTS

The results of our determinations of the ACTH contents of the hypophyses of 75 rats killed during the morning and the evening hours of the day are shown in Table and Fig. 1. The data of Table 1 show that the fall in ascorbic acid content of the adrenals of 7 groups of baby rats, following injection of extracts of rat hypophyses, was invariably greater (by 15-62 mg-%) when the hypophyses were taken from rats killed in the evening than in the morning. This finding shows that the ACTH of the hypophysis was higher in the evening than in the morning.

In order to find out whether the diurnal fluctuations in ACTH content of the hypophysis depends on the alternation of nights and days, viz., on photoperiodicity, a group of rats was maintained for 30 days under conditions of continual illumination, while a second group was kept in darkness for the same time. The ACTH content of the hypophyses of the animals of these two groups was then determined, for the morning (9 a.m.) and evening hours. As appears from Table 1 and Fig. 1, the diurnal fluctuations in ACTH content of the hypophysis are abolished or smoothed out when the animals are kept for a long time under conditions excluding the normal alternation of light and darkness. We have thus found experimental evidence of the dependence of the diurnal rhythm of the ACTH content of the hypophysis of rats on photoperiodicity of the environment.

It is known that the ascorbic acid content of the adrenals varies according to the functional state of the gland. The effect of ACTH in enhancing adrenal function is associated with a fall in its ascorbic acid content. It was therefore thought to be of interest to find out whether there existed a diurnal rhythm in the ascorbic acid content of the adrenals of rats. We determined the ascorbic acid content of the adrenals of adult rats in the morning and the evening, by the method of Roe and Kuether. The times of killing the animals were the same as in the experiments for determination of ACTH content.

Table 2 and Fig. 2 present the results of such determinations in 9 groups of rats (120 animals in all). All groups showed considerable differences between the ascorbic acid content of the adrenals of rats killed in the morning or the evening. Higher contents (by 43-116 mg %) were always found in the evening. The ascorbic acid content of the adrenals thus undergoes regular diurnal fluctuations.

In order to correlate this rhythm with the photoperiodicity of the environment, we took 3 groups of 10 rats each, and exposed them to continual illumination, while another 3 groups were kept in continual darkness.

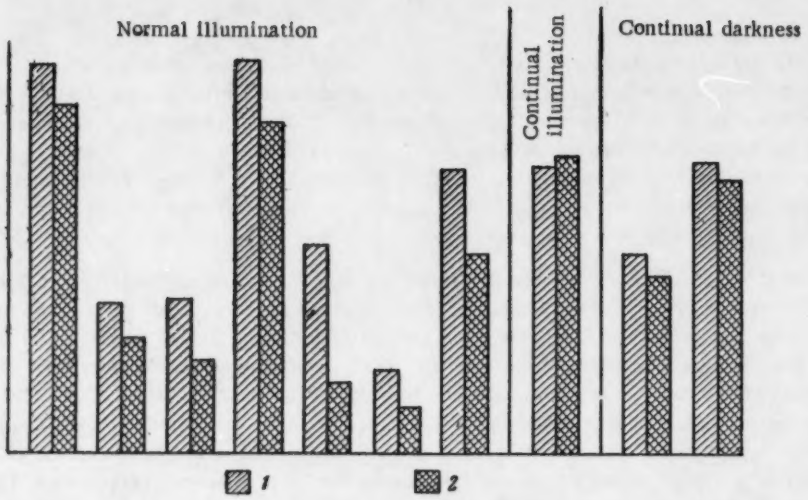

Fig. 1. Ascorbic acid content (in milligrams per 100 g) of the adrenals of newborn rats following injection of extracts of hypophyses of rats killed in the morning or evening. 1) Morning, 2) evening.

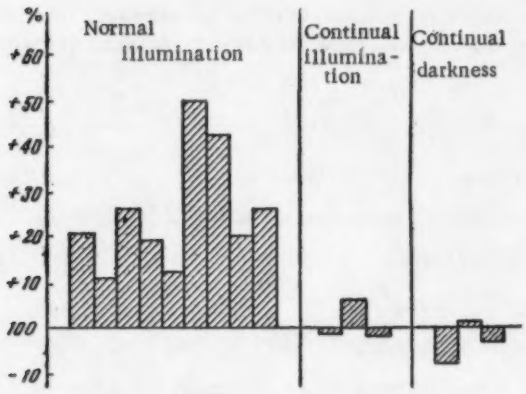

Fig. 2. Ascorbic acid content of the adrenals during the evening hours (as percentages of the values found during the morning).

After the animals had been maintained under these abnormal conditions of illumination for 30 days the rhythmic fluctuations in ascorbic acid content were found to have been abolished. It may be concluded that the diurnal rhythm in ascorbic acid content of the adrenals of rats is also dependent on regular photoperiodicity.

TABLE 2

Ascorbic Acid Content of the Adrenals of Adult Rats at Different Times of the Day

Experimental conditions	Ascorbic acid content (mg-%)		Change in ascorbic acid content (mg-%)
	morning	evening	
Normal illumination	338	409	+71
	425	472	+47
	368	465	+97
	279	331	+52
	352	395	+43
	232	348	+116
	232	329	+97
	388	466	+78
	336	422	+86
Continual illumination	383	379	—4
	394	418	+24
	366	358	—8
Continual darkness	385	356	—29
	403	409	+6
	440	429	—11

It is of interest that the ascorbic acid content of the adrenals was found to be higher in the evening than in the morning. This finding would at first sight seem not be in agreement with the enhanced adrenocorticotrophic function of the hypophysis during the evening hours, and with the earlier evidence of the fall in eosinophil count of peripheral blood, and of the rise in urinary 17-ketosteroid excretion in the evening. This discordance is, however, only an apparent one. The lowering of the ascorbic acid content of the adrenals in a

short-term response to a single dose of ACTH, or to the action of a stress-producing agent. The transient fall in ascorbic acid content is followed by its regeneration. Prolonged administration of ACTH (Comsa and Leroux, 1955), or maintenance of chronic stress conditions (Endröczi, Lassak, and Szereday, 1956), are associated with elevation of the ascorbic acid content of the adrenals. The same is the case in the evening, when the adrenals have been exposed to prolonged stimulation by ACTH secreted during the day. As with multiple doses of ACTH, one would expect to find an increase, rather than a fall, in the ascorbic acid content of the adrenals in these circumstances, and this is in fact what takes place.

Our results permit of the conclusion that there is a definite diurnal rhythmicity of adrenocorticotrophic function of the hypophysis, and of corticoadrenal function, regulated by the effect of light, i.e., of photoperiodicity. Expressions of these rhythms are to be seen in the diurnal fluctuations of ACTH content of the hypophysis, of ascorbic acid content of the adrenals, of 17-ketosteroids in the urine, of the 17-keto- and 17-hydroxycorticosteroids contents of the blood and urine, and of the total leucocyte, eosinophil , and lymphocyte counts of peripheral blood. It is probable that, as with the relations between the gonads and the gonadotrophic function of the hypophysis (Bissonette, 1936; Benoit, 1938; Ia. M. Kabak and S. I. Tereza, 1939, and others), or with the relation of thyroid activity to thyrotrophic activity of the hypophysis (Voitkevich, 1945, and others), light acts through photoreceptors on the hypothalamic region to enhance the adrenocorticotrophic activity of the hypophysis, and thus to stimulate the function of the adrenal cortex.

SUMMARY

The ACTH content of the hypophysis of rats rises from a minimum in the morning to a maximum in the evening hours. These fluctuations are associated with the diurnal rhythm of light and darkness, and are abolished under conditions of continual light or darkness for 30 days. Rhythmic fluctuations of the ascorbic acid content of the adrenals are also found, with maximum values in the evening, and these are likewise abolished by maintenance under conditions of constant illumination.

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THE EFFECTS OF ADRENALIN ON MITOSIS, RESPIRATION AND GLYCOLYSIS IN THE CORNEA AND INTESTINAL MUCOSA OF RATS

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The question of the effect of adrenalin on the mitotic activity of tissues in the organism was dealt with extensively in the experimental works of a number of authors.

In experiments involving removal and subsequent reimplantation of the suprarenals [2, 5, 8] and injection of a solution of adrenalin, it was shown that adrenalin inhibits mitotic division in the epithelium of the cornea, tongue, duodenum and epidermis and does not effect appreciably mitoses in cells of lymph nodes, thyroid, uterus and metastatic or recurrent tumors [1-4]. Adrenalin does not effect the mitotic activity of the epithelium of the small intestine either [8].

In view of the fact that much data found in the literature points to the intimate relationship between cellular growth and division, and carbohydrate – phosphorus metabolism, it was expedient to investigate the effect of adrenalin on the processes of respiration and glycolysis in various tissues, taking into consideration the definite role of adrenalin in these processes. It is known that in the presence of intense cellular division aerobic glycolysis or incomplete oxidation of carbohydrates, producing not only the necessary energy for the cells but plastic material as well, is quite typical for the tissues of the organism.

The purpose of this work was the comparative study of the effect of adrenalin on cellular division, aerobic glycolysis and consumption of oxygen in the cornea and the epithelium of the small intestine of rats.

EXPERIMENTAL METHOD

Investigations were carried out on white male rats weighing 150-200 g. Tissues were fixed in Bouin's solution prior to histological examination. 200-250 visual fields were examined in each preparation of the entire cornea stained with Carazzi's hematoxylin – eosin. A segment of the small intestine (10 cm from its gastric junction) was sectioned 8 μ in thickness after it was embedded in paraffin. In each case mitoses were counted in 50 crypts. In part of a crypt the total number of cells was counted.

In the biochemical investigations the corneas were dissected from other tissues of the eye along their edges in a cold 0.9% solution of NaCl immediately after the rats were killed. After drying the corneas with filter paper they were cut in half, weighed on torsion scales and placed immediately into an incubating medium. The corneas from left eyes (30-40 mg) were used for tests prior to incubation and from right eyes for tests after incubation. The mucosa of the small intestine was obtained by scraping in the cold with a glass slide with polished edges [11]. The tissue was gently stirred with a scalpel, weighed on torsion scales and promptly placed in the incubating medium. The rate of glycolysis was determined by the increase in the amount of lactic acid after incubation. Incubation was carried out for 1 hour in a Warburg vessel at air temperature of 37°. A 1.2 ml (pH = 7.4) freshly prepared solution [90 ml of 0.9% solution of NaCl, 8 ml solution of Na_2HPO_4 (0.4 M), 2 ml of solution of KH_2PO_4 (0.4 M) and 100 mg of glucose] was used as an incubating medium. Oxygen

absorption was measured manometrically, the lactic acid concentration – colorimetrically using p– oxydiphenyl following the method of Barker and Sammerson modified by S. L. Bonting. Proteins were precipitated with hot acetic acid. In order to make extraction of acetic acid more complete the contents of the Warburg vessel was placed into centrifuge tubes, the corneas macerated with a glass rod and the tubes allowed to stand for 10-15 minutes. After the material was centrifuged and 2 ml of corneal filtrate or 1 ml of intestinal filtrate obtained, 0.2 ml of 10% solution of NaCl was added to the former or 0.1 ml to the latter; the following were added to either: 0.5 ml of 20% of $CuSO_4$, 0.5 g CaO and distilled water to make 5 ml. The concentration of lactic acid was determined electrophotocolorimetrically. The quantity of oxygen absorbed was expressed in cubic millimeters to 100 mg of wet weight (QO_2), the concentration of lactic acid – in v/mg of wet tissue weight.

EXPERIMENTAL RESULTS

Data on the measurement of the mitotic coefficient in the cornea and intestine summarized in Table 1 shows that when an intramuscular injection of adrenalin is given (2.5 mg/kg of animal weight), a significant lowering of the mitotic coefficient in the corneal epithelium of rats takes place and lasts not less than 12 hours after injection. Forty-five minutes after injection of adrenalin the ratio of the absolute number of early phases of mitosis (prophase and metaphase) to later phases (anaphase and telophase) is equal to 1.3; in the corneas of rats sacrificed in 3 hours this ratio is equal to 2.2. In control animals which were given an injection into the soft tissues of the thigh and in all experimental animals sacrificed later on this ratio, with rare exceptions, was close to 3.0.

TABLE 1

Average Mitotic Coefficient in the Epithelium of the Cornea and Crypts of the Intestine at Different Periods Following Injection of Adrenalin and in Control Rats

Adrenalin injections			Soft tissue puncture		
period of sacrifice after injection	mitotic coefficient (in ‰)		period of sacrifice after puncture	mitotic coefficient (in ‰)	
	cornea	intestine		cornea	intestine
45 minutes	3,7	25,9	45 minutes	9,9	42,4
3 hours	2,2	48,1	—		
7 "	2,5	31,2	—		
12 "	1,3	35,4	12 hours	9,6	38,6
18 "	16,9	31,8	—		
1 day	8,7	28,3	1 day	13,4	32,5
2 days	17,6	36,7			

Entirely different results were obtained in the case of the epithelium of the crypts of the small intestine. The lowest mitotic coefficient (25.9‰) was obtained in rats sacrificed 45 minutes after adrenalin injection. For all subsequent periods the mitotic coefficient, in experimental as well as control rats, remained fairly high and not subject to any regular changes. Neither were any essential differences observed in the ratio between the absolute amount of early phases of mitosis and later phases.

In order to make the data obtained more precise a second series of experiments was carried out in which, besides adrenalin injection, injections of colchicine were also given. The animals in this group were divided into 4 groups with 7-8 rats in each. The rats were sacrificed $5\frac{1}{2}$ hours after injection. The results from the study of mitotic coefficients presented in Table 2 indicate that injections of 1.3 mg of adrenalin per 1 kg weight also results in abrupt lowering of the mitotic coefficient in corneal epithelium and does not cause lowering in the number of mitoses in the epithelium of intestinal crypts.

The results of experiments with subcutaneous injection of colchicine (0.2 mg per 150 g body weight) were unexpected. Typical pictures of metaphase arrested by colchicine were observed in the corneal epithelium of the animals in this group. In some animals the amount of mitosis was insignificant and for this reason the average mitotic coefficient for the entire group was found to be even lower than in controls. The number of mitoses in

the stage of metaphase increased in the epithelium of intestinal crypts. In those animals which were given simultaneously an injection of colchicine in one extremity and adrenalin in the other, the mitotic coefficient in the corneal epithelium was very low (3.2‰) while that in the intestine was unusually high (248.3 ‰).

TABLE 2

Average Mitotic Coefficient (in ‰) in the Epithelium of the Cornea and Crypts of the Small Intestine of Rats

Experiment No.	Substances administered	Mitotic coefficient	
		corneal epithelium	epithelium of intestinal crypts
1	Physiological salt solution	12.5	32.0
2	Adrenalin	2.7	30.2
3	Colchicine	11.0	211.5
4	Colchicine and adrenalin	3.2	248.3

Thus, the experimental data obtained indicates that adrenalin depresses the entry of the epithelial cells of the cornea into mitotic division and does not influence significantly mitotic division of the epithelial cells in the intestinal crypts.

TABLE 3

Effect of Adrenalin on Aerobic Glycolytic Action and Oxygen Consumption in the Cornea of Rats

Expt. No.	Control				Experiment			
	Q_{O_2} (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/mg of raw weight			Q_{O_2} (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/mg of raw weight		
		prior to incubation	after incubation	difference		prior to incubation	after incubation	difference
1	24,20	0,371	1,823	1,452	21,90	0,522	2,03	1,508
2	17,21	0,00	1,06	1,06	13,57	0,150	1,14	0,99
3	18,63	0,341	2,02	1,679	21,66	0,60	2,45	1,85
4	16,28	0,154	3,22	3,066	17,80	0,142	3,58	3,438
5	10,03	0,451	2,973	2,522	15,99	1,038	2,41	1,372
6	21,06	0,216	1,701	1,485	18,81	0,33	2,12	1,79
7[1]	23,65	1,056	2,564	1,508	26,66	0,933	2,744	1,811
Average	17,90	—	—	1,877	18,29	—	—	1,825

*Removal of corneas 30 minutes after injection of adrenalin (not taken into account in calculating averages).

In the first series of biochemical investigations of the effect of adrenalin on oxygen consumption and aerobic glycolysis in the rat cornea, intramuscular injections of adrenalin were given in the amounts of 1.3 mg per 1 kg body weight. Animals which were given 0.2 ml of 0.9% solution of NaCl served as controls. Corneas were excised 2 hours after injection during the period of maximal inhibition of mitotic division of epithelial cells.

Results of the experiments presented in Table 3 indicate that intramuscular introduction of adrenalin (1.3 mg per 1 kg body weight) does not produce a noticeable effect on oxygen consumption and lactic acid accumulation in the rat cornea under aerobic conditions. According to the data of experiment No. 7, no

noticeable changes in the indicated tests were demonstrable 30 minutes after the injection of adrenalin. However, we consider it premature to conclude that the effect of adrenalin on mitotic division of cells in corneal epithelium is not conditioned by its influence on the processes of respiration and glycolysis. Additional biochemical investigations using not only whole corneas but also a single layer of its epithelium are needed.

TABLE 4

Effect of Adrenalin on Aerobic Glycolytic Action and Oxygen Consumption in the Epithelium of the Small Intestine of Rats

Expt. No.	Control				Experiment			
	QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/ mg of raw weight			QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/ mg of raw weight		
		prior to incubation	after incubation	difference		prior to incubation	after incubation	difference
1	64,20	1,85	4,62	2,77	54,20	2,45	4,62	2,17
2	43,20	0,97	5,82	4,85	59,30	1,00	4,83	3,83
3	48,10	2,08	4,70	2,62	52,30	4,00	8,11	4,11
4	58,40	1,37	6,10	4,73	42,30	2,35	8,28	5,93
5	58,40	0,76	5,59	4,83	50,70	3,54	8,09	4,55
6	35,52	1,62	3,37	1,75	44,20	2,00	3,49	1,49
7	47,97	1,86	4,18	2,32	44,40	2,67	3,27	0,60
8	41,91	0,00	1,35	1,35	38,39	0,00	1,59	1,59
Average	49,34	—	—	3,15	48,22	—	—	3,03

The results of the second series of biochemical investigations carried out on the mucosa of the small intestine of rats whose mitotic activity does not change under the action of adrenalin are presented in Table 4. As one can see from this table, introduction of adrenalin intramuscularly does not produce any noticeable effect on oxygen consumption and lactic acid accumulation under aerobic conditions 2 hours after injection.

Thus, during the period of maximal inhibition of mitoses in corneal epithelium by adrenalin, oxygen consumption and lactic acid accumulation under aerobic conditions in the cornea as well as in the intestine remain unchanged. Further studies of the effect of adrenalin on individual aspects of metabolism in its relation to its varied action on mitotic activity of tissue are necessary.

SUMMARY

Intramuscular injection of adrenalin causes a significant decrease of mitotic activity in the cornea of rats and produces no effect on the epithelium of the crypts of the small intestine. Similar results were obtained after injection of adrenalin simultaneously with colchicine. During the period of maximal inhibition of mitoses in the corneal epithelium by adrenalin the consumption of oxygen and accumulation of lactic acid under aerobic conditions remain unchanged both in the cornea and in the intestine.

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MORPHOLOGY AND PATHOMORPHOLOGY

HISTOCHEMICAL STUDY OF THE GLYCOGEN CONTENT OF THE UTERUS AND PLACENTA OF WHITE RATS AT DIFFERENT STAGES OF GESTATION

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Our earlier histochemical studies [4] revealed certain regularities in the distribution of acid and alkaline phosphatase activities of uterine and placental tissues of white rats at different stages of pregnancy. The placenta of these animals is distinguished by its very high phosphatase activity, manifested by the high metabolic level prevailing in this tissue. The highest activities were found in the trophoblast and in its membrane surrounding the fetal vessels, and which is in contact with the maternal blood lacunae.

Bearing in mind the connection between carbohydrate metabolism and phosphatase activity, we undertook a study of the content and histotopography of glycogen in the uterus and placenta of rats at various stages of pregnancy, using histochemical methods for this purpose.

We have been able to trace only a very few papers dealing with the histochemical study of carbohydrate metabolism in the placenta. S. Kasab'ian [2] applied Shabadash's method to the investigation of glycogen content of the human placenta at various stages of pregnancy (1 to 10 months), and found a high glycogen content of the villi during the first three months, followed by an abrupt fall. The same was found for the decidual tissues. From his data, it appears that the process of glycogen storage proceeds most actively in the trophoblast. The active part played by the trophoblast in the carbohydrate metabolism of the ovum has been shown by Goldman [6], who was able, using Best's carmine method, to demonstrate the presence of glycogen in the trophoblastic syncytium of rat placenta. Wislocki, Deane, and Dempsey [10, 5] were not able to confirm this finding. These authors applied the Bauer-Feulgen, the Best carmine, and the Wislocki silver-staining methods [9] to the study of the content and the distribution of glycogen in the placentae of guinea pigs, white mice, rabbits, jerboas, and rats, without, unfortunately, sufficiently taking into account the stage of gestation at which the tissues were examined. These authors were able to detect glycogen only in the mononuclear giant cells of rodent trophoblast tissue, and could not find it in the trophoblastic syncytium. Other workers, apart from those cited above, have been unable to detect glycogen in the trophoblastic syncytium.

EXPERIMENTAL METHODS•

Our experimental material consisted of uteri and placentae taken from 18 white rats. The tissues were taken from nonpregnant animals, and from gravid rats on the 6th, 10th, 15th, 17th, 18th, and 19th days of pregnancy, and were fixed in a mixture of 9 parts of absolute alcohol and 1 part of neutralized formalin. Glycogen was stained by the Bauer-Feulgen procedure. We used sodium sulfite instead of sodium bisulfite for the

•The experimental material was supplied by Prof. P. G. Svetlov.

preparation of Feulgen's reagent and of the washing fluid. Paraffin tissue sections 6-8 μ in thickness, were smoothed down in a drop of reagent (20 g of potassium dichromate and 5 g of chromic acid in 1 liter of distilled water) on a slide previously treated with albumin-glycerol mixture. Some of the sections were straightened out in a drop of distilled water, but this did not give equally good results. The sections were cleared in the usual way, except that chromic anhydride was added to the 45% alcohol. The staining of the sections was always checked by ptyalin treatment.

EXPERIMENTAL RESULTS

Glycogen was found in traces in the non-gravid myometrium, in the apical parts of the epithelial cells of some of the endometrial glands, and in their secretion.

Glycogen could not be detected in the uterus on the 6th day of gestation, when implantation of the ova had not yet taken place [3], or was in progress. Only traces of glycogen could be seen in the myometrium.

By the 10th day, when the elements of the future placenta, assuring the further development of the fetus, had already been laid down, the uterus contained considerable amounts of glycogen. It was particularly abundant at the mesometrial side of the proliferating endometrium. The glycogen is seen in the form of grains and granules of different sizes in the cytoplasm of cells of the decidual tissues located between the larger lacunae

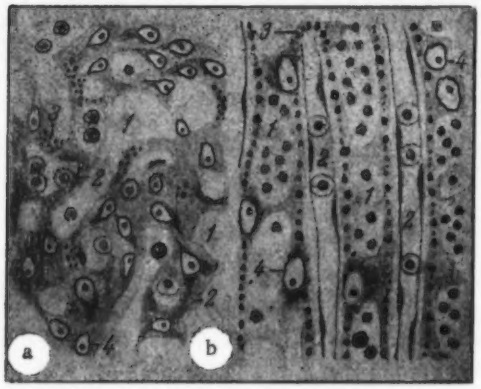

Fig. 1. Sections of white rat placentae (a) of the 15th, (b) of the 19th day of gestation: 1) subchorial blood space; 2) fetal capillaries; 3) glycogen in the trophoblastic syncytium (black spots); 4) syncytial nuclei. Magnification: Ocular 7 x, objective 40x.

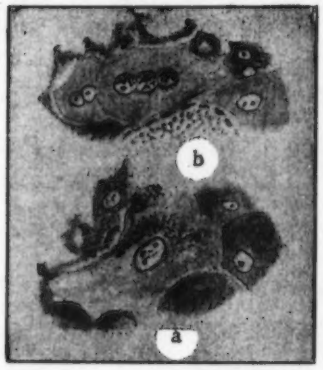

Fig. 2. Glycogen (black spots) in giant mono- and polynuclear cells of the trophoblast of the placentae of white rats, (a) on the 15th, (b) on the 19th day of gestation. Magnification: ocular 7x, objective 20x.

distributed around the ectoplacental cone. Its amount diminishes as it approaches the muscularis and the margin of the uterine cavity, where it can no longer be perceived. Only traces of glycogen were seen in the maternal epithelium, particularly over the fetal sac, where the cells are stretched considerably, and in the muscle layer. It was found in considerable amount in certain cells of the ectoplacental cone, which is derived from the trophoblast. Many of the cells did not contain glycogen, or showed only traces of it. Glycogen was not seen in the cells of the ectoplacental cone which were in a state of mitotic division.

The placenta is fully formed by the 15th day of gestation. At this stage, and thereafter up to full term, glycogen is absent from the allantoic mesenchyme zone of the placenta. Only in very rare cases can it be seen in the trophoblastic syncytium of the subchorial blood spaces, as minute droplets or granules (Fig. 1, a). Islets of maternal tissue consisting of more than 10 cells, as well as isolated maternal cells found in the blood spaces, are rich in glycogen. It is absent from the trophoblast of the boundary zone along the narrow lacunae of maternal blood, but is present in large amounts in the so-called "glycogen cells", situated in large groups between the lacunae. Many of these cells do not, however, contain any glycogen. Glycogen is visible in the form of aggregates of different sizes, in many of the giant cells (Fig. 2, a). It is concentrated in the peripheral cyto-

plasm of the cells, or in the perinuclear region, and only rarely is a giant cell uniformly packed with glycogen. Some of the giant cells contain a dark brown pigment, in the form of short, rod-shaped particles; this appears to be hemosiderin [11]. Such cells contain finely granular glycogen. Glycogen is distributed irregularly through the maternal part of the placenta. Large conglomerates of glycogen are present in the cells contiguous with the giant cell zone, where the larger venous sinuses are situated. Less glycogen is present, mostly in the form of granules and droplets of different sizes, toward the line of future separation of the placenta. Some parts of the maternal placenta do not contain any glycogen. Large globular cells, with a granular cytoplasm, are to be encountered among trophoblast cells forming the lining of the lumina of the venous sinuses; these cells are rich in glycogen. These cells, which often possess two nuclei, sometimes protrude into the lumen of the sinus, where they are separated from the maternal blood by a layer of attenuated endothelium. Granular glycogen-containing cells may be seen in other sections in the lumen of the venous sinuses. Glycogen-loaded granular cells, with one or two nuclei, are found in the greatest number around the blood vessels of the maternal placenta, and are even more numerous in the extraplacental part of the decidua basalis, which is the chief site of their formation. Single glycogen-loaded cells are to be found in the subchorial blood spaces, to which they were carried by the blood stream.

The granular cells, which were first described in rats by Gerard [6] in 1925, probably then disintegrate. Evidence in support of this is afforded by the presence among such cells seen in the lacunae of weakly staining forms, with barely distinguishable outlines. Moreover, in agreement with the observations of other authors [7], we found granules of free glycogen in the blood of the venous sinuses. There are no clear indications as to the origin and functions of the granular cells. Endocrine, phagocytic, and trophic functions have been ascribed to them [6]. According to some workers, these cells originate from fibroblasts, while others believe that they originate from muscle or decidual cells, or that they are hypertrophied endothelial cells.

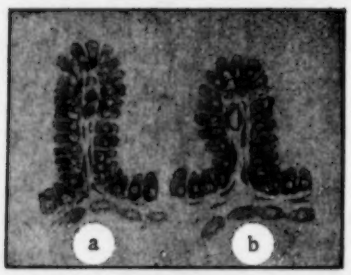

Fig. 3. Section of the wall of the yolk sac of (a) a 15-day, (b) a 19-day white rat fetus. Glycogen is seen as black spots in the entodermal epithelium. Magnification: ocular 7x, objective 20x.

In our opinion, these cells are of connective tissue origin, since on the 6th day of gestation cells containing acidophilic granules are to be found in the lamina propria, in the vicinity of the muscular coat, and it is probable that it is from these that the glycogen-rich, globular, granular cells originate. Glycogen is present in small amount in the myometrium. It is absent from the cells of the walls of the yolk sac, or is present in traces or small amounts only (Fig. 3, a).

The placenta achieves its highest degree of development on the 17th day of gestation. at this stage, the islets of maternal tissue found in the placental labyrinth zone are loaded with glycogen. The glycogen content of the attenuated syncytial trophoblast rises. It is distributed as very fine granules throughout the whole of the labyrinth zone. Its distribution in the other elements of the placenta remains similar to that found on the 15th day of pregnancy. In the giant cells, the glycogen is frequently concentrated in one particular part of the cell. The glycogen content of the maternal placenta falls. It may be found in the walls of the umbilical artery up to the termination of pregnancy, and in smaller amount in the umbilical vein. Glycogen is absent, or present only in traces, in the folded part of the wall of the yolk sac. It is absent from many cells. It is concentrated mostly in the basal, less frequently in the apical, regions of entodermal cells. It is absent from the cells of the smooth part of the wall of the yolk sac.

The glycogen content of the syncytial trophoblast of the labyrinth continues to rise during the 18th and 19th days (see Fig. 1, b), both the number and the dimensions of the granules increasing. Isolated conglomerates of glycogen may even be seen on the 19th day. A considerable part of the glycogen cells of the boundary zone have undergone degeneration at this stage. Glycogen is to be found in small amount in the trophoblast cells of certain regions of this zone. Little glycogen can be seen in the cytoplasm of the giant cells, being sometimes concentrated at the nuclear membrane (see Fig. 2, b). The glycogen content of the decidua basalis

remains high, being mostly localized in the granular cells. There is little glycogen in the epithelium of the folded parts of the wall of the yolk sac, being situated in the basal parts of the cells; many cells do not contain any (see Fig. 3, b). The glycogen content of the maternal epithelium is low.

In general, accumulation of glycogen is seen to vary parallelly with the phosphatase activity, and in particular the acid phosphatase activity, of a given region of the placenta and uterus; this is in accordance with the findings of other authors [8].

Both glycogen and acid phosphatase make their appearance in the rat uterus toward the 10th day of pregnancy. With formation of the placenta, glycogen is initially found in large amount in its maternal portion, but the content falls as pregnancy proceeds. Alkaline phosphatase is absent during the entire period of gestation, while acid phosphatase activity, which is high initially, falls as pregnancy proceeds.

The glycogen content of the decidua basalis also appears to be correlated with the acid phosphatase activity. As pregnancy advances, both of these factors show a parallel rise.

Glycogen is to be found throughout pregnancy in the giant cells of the trophoblast, both mono- and polynuclear. These cells show a low alkaline, but a high acid phosphatase activity. Both alkaline and acid phosphatase activities were found to be very high in the syncytial trophoblast of the labyrinth. Glycogen makes its appearance here from the earliest stages of formation of the placenta, and its persists, in gradually increasing amount, as pregnancy proceeds. The vitelline placenta plays scarcely any part in the carbohydrate metabolism of the fetus. The most active part is played by the trophoblast, which has also been shown by Z. P. Zhemkova [1] to be very actively concerned in protein metabolism. It thus appears that embryonic development is fulfilled largely as a result of the various activities of the placental trophoblast.

SUMMARY

Histochemical studies of the distribution of glycogen in sections of the placenta and the uterus of white rats were made, using the Bauer-Feulgen technique, on material from the 6th, 10th, 15th, 17th, 18th, and 19th days of pregnancy. Only traces of glycogen were found in the non-gravid uterus. During pregnancy there is a correlation between the phosphatase, in particular the acid phosphatase, activities of uterine and placental tissues and their glycogen contents. A gradual rise is seen in the glycogen content of the decidua basalis, the syncytial trophoblast of the labyrinths, and the giant cells of the trophoblast, viz., in those components of the fetal adnexa which play the most important part in the carbohydrate metabolism of the embryo. Only traces of glycogen are to be seen in the vitelline placenta.

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SYNAPTIC CONNECTIONS BETWEEN THE NEURONS OF THE INTRAMURAL GANGLIA OF THE PELVIC ORGANS

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New experimental evidence has been obtained recently, supporting the view that closure of peripheral reflex arcs takes place within the ganglia of the peripheral segment of the autonomic nervous system.

The findings of a number of workers [3, 4, 5], as well as our own observations [8, 9, 10], have established that Dogel's type II cells, the functional significance of which was until very recently unknown, are in fact peripheral sensory neurons, as A. S. Dogel' himself supposed [2]. The results of the morphological studies of a number of authors [1, 3, 4, 5], as well as our own findings [7, 9, 10], have provided evidence that these cells are, through their dendrites, in synaptic connection with effector neurons, thus forming reflex arcs which are closed within the limits of the peripheral ganglia.

We have worked out a technique for the removal of the spinal cord of cats, with the object of investigating interneuronic connections in the peripheral autonomic nervous system. In our preceding communication [10] we presented evidence that intact synapses persist after a lapse of time sufficient for degeneration of preganglionic fibers of central origin in the solar plexus.

In the present communication we present the results of a study of interneuronic connections in ganglia innervating the pelvic organs, after their total decentralization. Such studies are of special interest inasmuch as many of these ganglia differ from those of the solar plexus in belonging to the parasympathetic branch of the autonomic nervous system.

EXPERIMENTAL METHODS

We applied the methylene blue and silver impregnation method of Kampos, and Cajal's pyridine method, to the investigation of the nerve ganglia of the urinary bladder, urethra, prostate gland, and uterine cervix of cats, at various times after removal of the spinal cord.

EXPERIMENTAL RESULTS

Our experimental material was derived from 18 cats, killed between the 12th and the 34th day after total ablation of the spinal cord below the level of the 6-7th thoracic segment. A second operation, involving division of the splanchnic nerves, with subdiaphragmatic vagotomy, and division of the lumbosacral trunk, was performed on 4 of the cats. We found that after a time such animals begin to feed themselves, actively to catch mice placed in front of them, try to leave their cage when the opportunity presents itself, and to wash themselves, etc.; they behave like quite normal animals. In most cases, the fluid motion change into formed stools by the 5-7th day; this is evidence of a certain normalization of the activities of the alimentary tract, including also partial evacuation of the rectum. Periodic release of small amounts of urine from the

overdistended bladder is also noted. In one of our first experiments, on a cat which was found to be pregnant, we evidently damaged a fetus during one of the routine toilet operations (emptying of the urinary bladder and the rectum). During the night, the cat aborted a fetus, together with some blood clots.

Similar cases have been reported in the literature [13], and provide strong evidience of the existence of peripheral reflex mechanisms affecting the activities of the uterus.

A superficial survey of our sections showed the presence in the ganglia of nerve cells which could, from their dimensions, morphological features, and staining properties, be recognized beyond doubt as being Dogel's type II cells. They are distinguished in the first place by their large size, which approaches that of the average cell of a dorsal root ganglion (30-50 μ), and in the second place by the intensity of their staining or silver impregnation, in contrast to the weakly stained small cells (Fig. 1). There are several score small cells to each Dogel's type II cell seen in sections of large ganglia.

A characteristic feature of the small ganglia, made up of 10-15 small cells, is that there is usually a single large, intensively staining cell present. We noticed that such cells seen in preparations of the ganglia of the urinary bladder, of the cervix, and of the prostate gland differed somewhat in the nature of their dendrites from the Dogel's type II cells of the alimentary tract. As a general rule, the majority of the dendrites do not extend beyond the ganglion, but remain in intimate connection with the small cells of the ganglion.

Synaptic terminations are to be seen in all the above nerve ganglia, including also the intramural ganglia of the colon and rectum, at the motor nerve cells, in the form of very fine pericellular nerve fibrils entwined around the cells.

The nerve fibrils of which the synaptic apparatus is made up do not as a rule terminate around a single cell, but proceed further to other adjacent cells. Another characteristic feature is that this pericellular apparatus is usually to be encountered around cells situated toward the periphery of a ganglion, as has been previously found in the solar plexus.

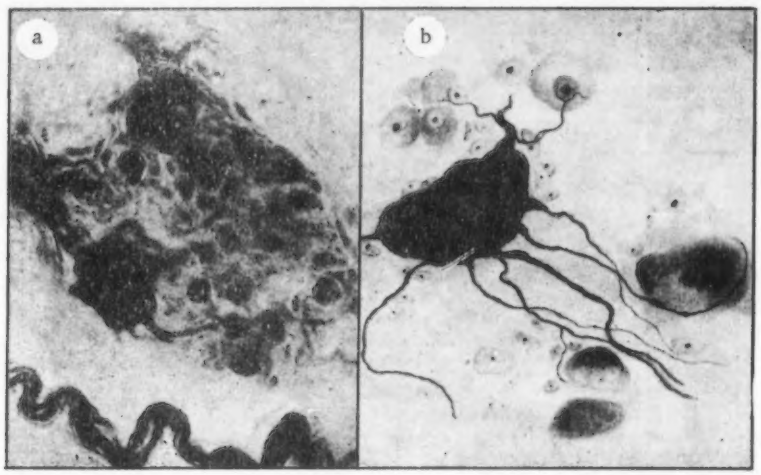

Fig. 1. Single sensory neurons in small ganglia: a) in a ganglion of the uterine cervix; b) in a ganglion of the urinary bladder (sketches of preparations).

Similar synaptic structures, made up of ultrafine pericellular nerve fibrils entwined around nerve cells, were also found in the intramural ganglia of the urinary bladder and of the cervix (Fig. 2). This type of synaptic apparatus exists not only in ganglia, but also at isolated nerve cells.

The form of synaptic apparatus described above is characteristic of the ganglia of animal from which the spinal cord has been removed.

In no cases did we find any synaptic structures in the forms of loops or rings, such as have been described by numerous authors for the synapses of the autonomic nervous system. Our results afford additional support for our previously expressed view that peripheral sensory neurons enter, through their axons, into synaptic connection with effector neurons, forming peripheral reflex arcs.

Morphologically, the synapses formed by the dendrites of Dogel's type II cells represent a system of pericellular nerve fibrils, belonging, according to W. Kirsche's classification [14], to the class of synapses "with a large field of transmission".

Special attention needs to be paid to the connections between neurons effected by dendrites of large cells, terminating within the same ganglion. As is known from A. S. Dogel's classical description [2], cells of ganglia of the autonomic nervous system with dendrites which ramify within the same ganglion within which the cell is situated are classified as type III cells. Similar cells have been described by numerous authors, in particular by F. de Castro [12], as being in intimate connection, through their processes, with other cells of the same ganglion, entwining their bodies and dendrites; this description corresponds very closely with that of A. S. Dogel'. Similar formations have recently been found in ganglia of the human autonomic nervous system by N. G. Kolosov [5] and his collaborator Yu. I. Slepkov [11]. These connections are particularly well defined in the ganglia of large animals and of humans (Fig. 3).

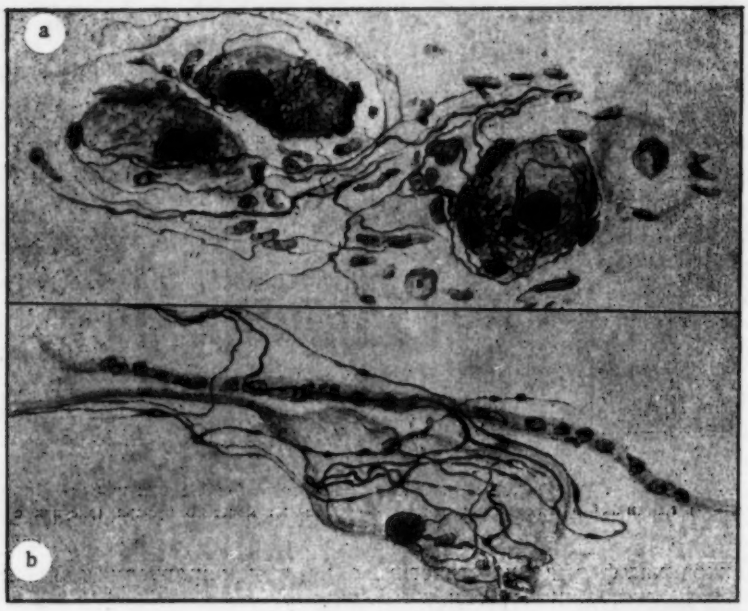

Fig. 2. Pericellular structures: a) in the uterine cervical gland (25 days after removal of the spinal cord, and 13 days after division of the splanchnic nerves, subdiaphragmatic vagotomy, and transection of the sacrolumbar trunk); b) pericellular apparatus of a single nerve cell of the urinary bladder (20 days after removal of the spinal cord, and 10 days after division of the splanchnic nerves and subdiaphragmatic vagotomy).

Different authors have ascribed different functions to cells of this type. A. S. Dogel', on the basis of his concept of the reflex function of ganglia, considered them to be associative neurons. F. de Castro [12] considers them to be effector neurons, and he regards the connections between the dendrites of these cells and of other neurons as being merely an adaptation to the transmission of nerve impulses from one preganglionic fiber to a group of effector neurons within the ganglion.

Iu. I. Slepkov [11] believes that these cells are identical with those of Dogel's type II, i.e., they are sensory neurons, forming receptor terminations at the other neurons. N. G. Kolosov [5] regards them as motor cells, although he also endows them with sensory neuron functions.

Such divergent views as to the nature of these cells, and to the physiological function of the connections between their processes and those of other nerve cells may be ascribed to the circumstance that, as is usually the case, the shorter processes which ramify within the ganglion itself are regarded as dendrites, i.e., as processes receiving nerve impulses. However, the classification of nerve cell processes as dendrites or axons is highly arbitrary. Sensory neurons, in particular, are distinguished by the circumstance that the thicker and longer peripheral process has a receptor function, i.e., it is a dendrite, while that proceeding to the brain is shorter and is highly ramified within the brain, but is an axon.

It is in most cases not possible to distinguish an axon from a dendrite of a Dogel's type II cell of the alimentary tract, the sensory nature of which is now beyond dispute. Moreover, the cell processes under discussion are not true dendrites, i.e., protoplasmic processes, but should rather be regarded as processes of the axonal type.

Fig. 3. Synaptic connections between neurons of a peripheral ganglion of the autonomic nervous system (stellate ganglion) of a bull (sketch made from a preparation).

We therefore believe that the contact between the processes of the large intensively staining cells and of the small cells of the ganglia is a real synaptic connection, being the morphological expression of the functional connection between peripheral sensory and effector neurons. In other words, such cases represent a reflex arc, of which the afferent limb is the Dogel's type II cells.

The circumstance that in some cases the processes of the sensory neurons (Dogel's type II cells) emerge from the ganglia, while in other cases they terminate within the ganglion, forming synaptic connections with its cells is evidence merely of special features of the construction of peripheral reflex mechanisms, depending on the functional peculiarities of a given organ.

It is evidently necessary, in order to perform a function of the complexity of intestinal peristalsis, to have nervous connection between remote ganglia of the intramural plexus. Such connections may be achieved by the long processes of the Dogel' type II cells, which emerge from the ganglion within which the cell is located.

In the more compact uterine cervical ganglia, and in those of the prostate gland and the urinary bladder, the sensory cells enter into synaptic connection with other cells of the same ganglion, by means of their

processes; for this reason the corresponding processes are shorter, and this determines the characteristic form of the sensory neuron.

SUMMARY

The functions of the intestine are not totally abolished following removal of the spinal cord of cats, below the level of the 6-7th thoracic segment. Evacuation of formed stools takes place, on a limited scale. Retention of urine is accompanied by periodic voiding of small amounts from the overdistended bladder. An injured fetus was spontaneously expelled from the uterus of one of the cats, showing that uterine function persisted after complete separation from the central nervous system. Synaptic connections could be demonstrated in the peripheral ganglia of the pelvic organs (bladder, urethra, prostate, cervix uteri), between sensory neurons (Dogel's type II cells) and effector neurons of the same ganglion.

Peripheral reflex arcs, with which the different limbs is provided by Dogel' type II cells, may thus render possible the functioning of viscera following the total severance of connections with the central nervous system.

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RELATION BETWEEN DEVELOPMENT OF FUNCTIONS AND THE STRUCTURE OF THE CEREBRAL CORTEX IN ONTOGENESIS

COMMUNICATION II. HISTOLOGICAL DIFFERENTIATION OF CORTICAL CELLS OF RABBITS DURING ONTOGENESIS.

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In Part I of this series [2] we presented data on the time required for formation of condition reflexes to various stimuli during ontogenesis in the rabbit. Histological studies were conducted parallel with the physiological ones. The histological part of these studies, which is the subject of the present communication, concerned the histological differentiation of nerve cells of the cerebral cortex and of a number of subcortical formations, covering the period from the 23rd day of intra-uterine existence up to the adult state. Some of the animals involved had been used for the physiological investigations.

EXPERIMENTAL METHODS

We followed two main lines in our research the study of the development of the structure of the cytoplasm of the cell bodies, and the study of the development of their processes, in particular of their synaptic formations. The study of cytoplasmic structures was made with the aid of Nissl's staining procedures and of staining of ribonucleic acid (RNA; staining according to Unna-Pappenheim, with Brachet's control technique). The study of changes in RNA content of nerve cells is of special interest, inasmuch as a number of authors, in particular, H. Hiden, have demonstrated the importance of RNA in the development and functioning of nerve cells. Golgi's silver impregnation method was applied to the study of cell processes and of their synaptic terminations. The results were recorded by means of photomicrographs.

EXPERIMENTAL RESULTS

The series of complex changes taking place during the process of differentiation of a nerve cell from the neuroblast stage to that of the mature neuron involves both qualitative and quantitative changes. For our purposes, special interest was attached to the qualitative changes, involving the appearance within the cells of new structures. Our object was to describe these qualitative changes occurring during the development of a nerve cell, and on this basis to arrange them in the sequence followed during differentiation of the cell.

We have, on the basis of our results and of published work, divided the process of differentiation of the internal structure of the cytoplasm of a neuron into the following 3 stages (Nissl and RNA staining; Fig. 1).

1st Stage. The cell has a deeply staining nucleus and a homogeneous nucleolus. The cytoplasm is scanty, and stains very weakly. No cell processes are evident.

2nd Stage. The cell nucleus is translucent. The nucleolus is homogeneous, but is larger than in Stage 1. The basophil cytoplasm stains diffusely (a foamy appearance in thin sections). Protuberances are evident at the sites of future processes (Fig. 2).

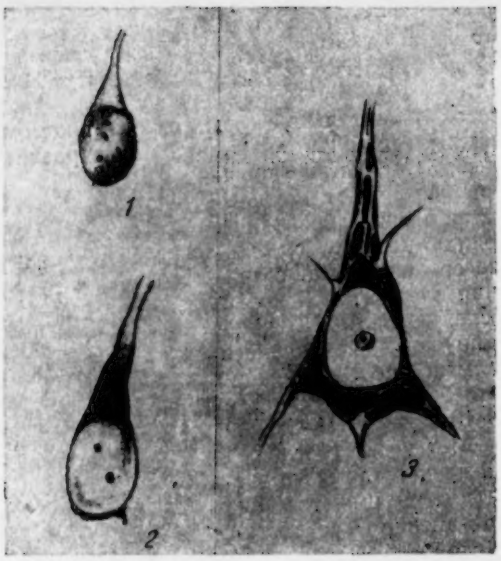

Fig. 1. Stages in the differentiation of the internal structure of the cytoplasm of a nerve cell (Nissl and ribonucleic acid staining). The figures refer to stages of differentiation.

3rd Stage. The cell nucleus is clear. The nucleolus contains crystalloid. The amount of cytoplasm shows considerable increase, and it is filled with well-defined clumps of tigroid. The cell processes are evident.

Fig. 2. Pyramidal cells from layer III of the cerebral cortex of a 6-day-old rabbit. Second stage of differentiation. Temporal region. Magnification 1200 ×.

Our data on the maturation of nerve cells of the cerebral cortex are presented in Table 1, adopting the above classification of developmental stages.

From these results we may draw the following conclusions.

1. The nerve cells of the cortex and subcortical parts of the brain all pass through the above 3 stages; our division of the process of differentiation of the neurons is therefore justified.

2. Cells belonging to different formations mature at different rates. The subcortical cells are already mature at the birth of the animal (we studied the development of cells of the striopallidus system and of the nuclei of the 7th pair of cranial nerves). Most of the cells of the rhinencephalon have achieved maturity by birth, and the remaining ones complete their development within two days of birth (Fig. 3). The cells of the neencephalon mature later than do those of the rhinencephalon. Maturity is achieved in the cells of the neencephalon by the following times: Layer I – Cajal – Retzius cells, which are found to be mature in a 23-day embryo, layer II – by the 10-13th day after birth, layer III by the 10-13th day, and layer IV by the 13th day (isolated mature cells are to be seen in 10-day-old rabbits), layer V by the 6-8th day, and layer VI by the 8th day. Differentiation of the cells of the

TABLE 1

Development of Internal Structure of the Cytoplasm of Nerve Cells During Ontogenesis (stained by Nissl's method and for RNA)

Layer and part of brain	Age, days										
	23-day embryo	0	2	5–8	8	10	13	15–16	25	40	adult
Neencephalon											
I	3	3	3	3	3	isol. 3	isol. 3	—	—	—	—
II	1	1 (2)	1 (2)	2 (1)	2 (1)	2 (1,3)	3 (2)	3 (isol.2)	3	3	3
III	1	1 (2)	1 (2)	2 (1,isol. 2)	2 (1,3)	2,3 (isol.1)	3 (isol.2)	3	3	3	3
IV	1	1	1 (isol.2)	1 (2)	1 (2)	1,2 (isol. 3)	3 (2)	3 (2)	3 (isol.2)	3	3
V	2,1	2 (1, isol. 3)	2 (1, isol. 3)	2 (3)	3 (2)	3	3	3	3	3	3
VI	2,1	2,1 (isol. 3)	2,1 (isol. 3)	2 (isol 3)	3 (2)	3 (2)	3	3	3	3	3
Rhinence-phalon	1,2	3,2	3	3	3	3	3	3	3	3	3
Subcortical	1,2	3	3	3	3	3	3	3	3	3	3

Note: The figures refer to stages of differentiation; those within parentheses signify that a minority of the cells are not the given stage; figures preceded by "isol" mean that only isolated cells are at the given stage.

neencephalon is thus basically completed by the 13th day post partum, although all layers contain mature cells by the 10th day.

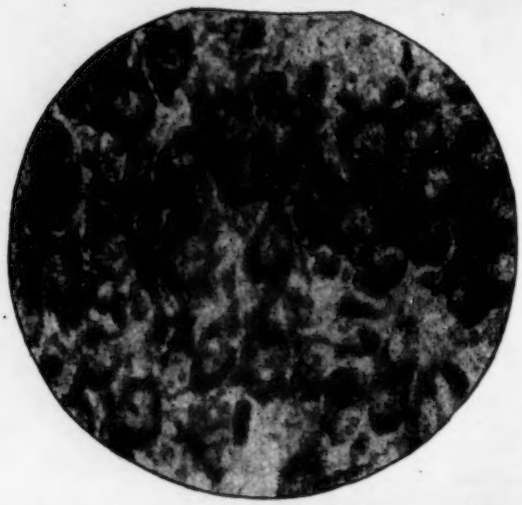

Fig. 3. Cells of the rhinencephalon of a 2-day-old rabbit. Third stage of differentiation. Entorhinal area. Magnification 600 ×.

The different times required for achievement of maturation of cells of different parts of the brain and of different layers of the neencephalon are in accordance with the general biogenetic rule that structures which are phylogenetically of more recent origin develop later during ontogenesis.

The cerebral cortex is phylogenetically more recent than are the subcortical formations, and it accordingly develops later in the course of ontogenesis.

The rhinencephalon is phylogenetically older than the neencephalon and accordingly its cells mature before those of the neencephalon (see Figs. 2 and 3). The lower layers of the neecephalon are phylogenetically older than the upper layers [2], and during ontogenesis their cells mature earlier than do those of the latter. The differences between the times of maturation of different parts of the neencephalon are insignificant. The greatest difference is in the somewhat later time of maturation (by 2-3 days) of the occipital regions.

Certain deviations from the biogenetic rule governing the general sequence of maturation of the cells of different parts of the brain do, however, occur, some individual formations maturing earlier than would be expected.

3. Individual nerve cells belonging to the same part and the same layer of the neencephalon may mature at different times. These differences are, however, much smaller than are the differences between times of maturation of cells of different types.

4. We have, on the basis of the data of Table 1, attempted to make a rough estimate of the duration of the process of maturation of a neuron. Since a cell may remain in its 1st stage of development for a considerable time without showing any significant structural changes, we took the duration of the differentiation process as being the time interval between entry of a cell into the 2nd stage of development and completion of differentiation (transition to the 3rd stage), i.e., as being equal to the duration of the 2nd stage of development. We thus obtained the following approximate values for the duration of the process of differentiation of a neuron (the data relate only to cells of the neencephalon): layer II 4-7 days, layer III 4-7 days, layer IV 3 days, layer V 16 days, layer VI 16 days.

A correlation can be perceived between the duration of the maturation process and the length of the axon of a given cell; thus it is known that the axons of layer IV cells are confined to the cortex, those of layers II and III form the association pathways, and those of layer V and VI are descending projection fibers, and are hence of considerable length.

Our conclusions apply equally to sections stained according to Nissl and for RNA. However, RNA staining permits one to draw a number of additional conclusions. Although staining of RNA does not give as fine detail of the internal structure of the cytoplasm as does staining according to Nissl, it has the advantage of giving greater contrast; it is of particular value in the study of the process of cell differentiation, since the difference in deepness of staining between mature and immature cells is quite considerable.

This staining procedure is also convenient for the study of processes taking place within the cell nuclei.

We found that the RNA content of the cells rose steeply during the process of differentiation. Calculations based on published biochemical data [5] and on our data for increase in the weight of the brain showed that

from the 23rd day of intra-uterine life to adult maturity there is a 40-fold increase in the average RNA content of a cell. There is a particularly large increase in the RNA content of the cytoplasm at the time of transition of a cell into its 3rd stage of maturity. Changes in the distribution of RNA in the cytoplasm also take place during the process of differentiation. During the 2nd stage of maturity RNA is diffusely distributed throughout the cytoplasm, whereas in the fully mature cell it is concentrated into clumps of tigroid.

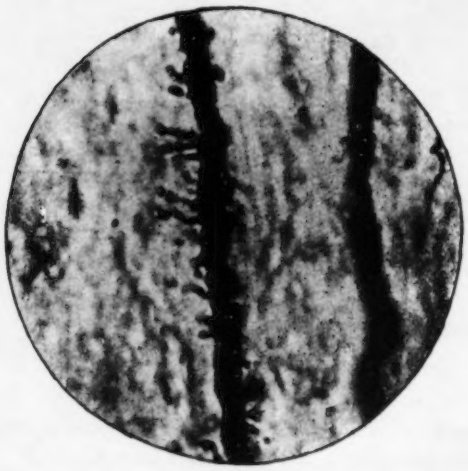

Fig. 4. Lateral outgrowths (spines) on a dendrite of a cortical cell of a 19-day-old rabbit. Third stage. Magnification 1200 ×.

The staining of the nucleus changes during the process of differentiation. The nucleus of an immature cell has a high chromatin content, with diffusely distributed RNA. The intensity of staining then diminishes, until in the mature cell it is practically colorless.

Due to the distribution of nucleolar chromatin, particularly striking changes take place in the nucleolus, which develops from the stage of being a small, homogeneous body to that of a large, RNA-rich nucleolus with a well-defined structure. The number of nucleoli falls during the process of differentiation from 3-4 to 1. A connection may be discerned between the changes in nucleolar structure and the rise in their RNA content with the rise in the RNA content of the cytoplasm, and the formation of Nissl bodies.

In studying the development of the dendrites of nerve cells and of their synaptic terminations we paid special attention to the development of the so-called "dendritic spines," which are the morphological substrate of axodendritic synaptic connections [3, 4, 5]. The dendritic spines are most highly developed in the pyramidal cells of the neencephalon, and somewhat less so in the cells of the olfactory cortex; the cells of subcortical formations possess far fewer spines than do those of the cortex. We also divided the process of cell differentiation as shown by Golgi staining into 3 successive stages. For this purpose we made use of A. D. Zurabashvili's scheme [1] for cells of the spinal cord. We introduced a few modifications into this scheme, so as to adapt it to the cells of the cerebral cortex.

We divided the process of cell differentiation into the following stages:

Stage I – cells with a weakly developed system of processes; in pyramidal cells only the apical dendrite is evident.

Stage II – appearance of branches of the 2nd or 3rd order in the apical dendrite, of basal dendrites, and of dendritic spines (subdivided into stage IIa, in which the spines are present only in the proximal part of the processes, and stage IIb, in which they are seen in the terminal arborizations of the dendrites).

Stage III – the mature nerve cell, with the arborization of a large number of processes; the dendritic formations are practically covered with spines (Fig. 4).

As was shown by further observations, these stages of differentiation of cell processes coincide largely with those of the cell body, as described above.

Our results are presented in Table 2, in which this scheme for the subdivision of the process of cell differentiation is incorporated. The following conclusions may be drawn from these results:

1) subcortical cells, and cells of the rhinencephalon have achived maturity before the termination of pregnancy, while those of the neencephalon achieve it within 10-15 days of birth;

2) a certain correlation is evident between the stages of differentiation of the cell body and of its processes; this may be an indication of the role of the cell body in the formation of its processes and synaptic terminations.

Thus the maturation of the cytoplasmic structures of the cell body and the maturation of its processes, with their synaptic terminations, proceed concurrently.

TABLE 2

Scheme of Development of the Processes of Cortical Cells and of their Synaptic Terminations

Layer and region	Age, days					
	1	5	10	15	30	adult
Neëncephalon III	I (IIa)	IIa	IIb	III	III	III
Neëncephalon V	IIa	IIa	IIa –III	III	III	III
Rhinencephalon	III	III	III	III	III	III
Subcortical	III	III	III	III	III	III

Note: The Roman numerals represent stages of differentiation.

The following conclusions emerge from our results:

1) We have subdivided the process of ontogenetic differentiation of neurons into 3 stages, each of which is distinguished by qualitative differences in the structure of the neuroplasm and of the cell processes.

2) The process of differentiation of a neuron is associated with a fall in the intensity of staining of the nucleus, with increase in RNA content and size of the nucleolus, with the accumulation of large amounts of RNA in the cytoplasm, with formation of clumps of tigroid, into which cytoplasmic RNA is concentrated, with increased arborization of dendrites, and with formation of dendritic spines.

3) The cells of different parts of the brain mature at different times. Most of the cells of subcortical formations and of the rhinencephalon are mature at birth; those of the neencephalon achieve maturity within 10-13 days of birth. The cells of different parts of the brain mature in a sequence which is in accordance with the biogenetic rule of ontogenesis.

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METHODS

IMPLANTATION OF ELECTRODES IN THE BRAIN OF WHITE RATS IN EXPERIMENTS OF LONG DURATION

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A method of implantation of electrodes in the brain for long periods of time has been described only in cases of comparatively large experimental animals – dogs, cats and rabbits [1, 2, 3]. It appeared desirable to devise a method of implanting electrodes for a long period of time in the brain of white rats, which are suitable subjects for many experimental investigations.

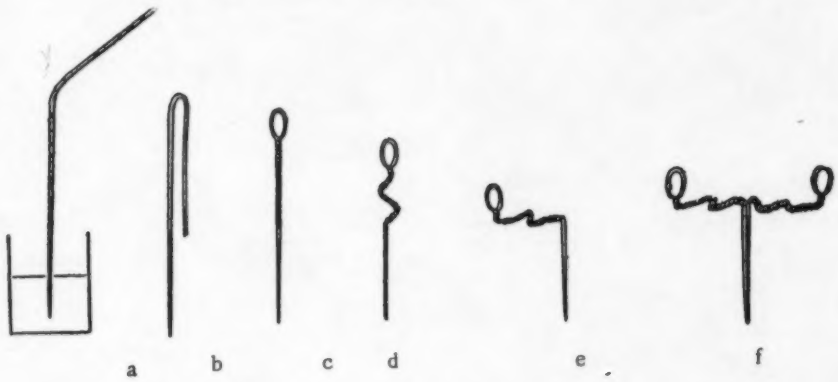

Fig. 1. General view of the electrodes and the stages in their preparation.

The essence of the suggested method consists of the creation of a firm base on the skull for attachment of the electrodes. For this purpose a specially prepared perforated plate, made of organic glass, to which the bipolar electrodes are attached, is affixed subcutaneously to the bones of the skull.

Preparation of the supporting perforated plate. A solution of organic glass in chloroform (1 : 1) is poured into a Petri dish so that after slow evaporation of the chloroform a plate no more than 1 mm in thickness was obtained. Next, cellophane is inserted between several prepared plates, which are then flooded with 96% alcohol. The plates stay very flexible and transparent for a long time. At the time of operation trapezoidal plates are cut out with scissors from these, corresponding in size to the area of the vault of the skull of the rats undergoing operation. Next the plate is perforated by means of a specially adapted cork-borer in order to obtain 4 oblong holes.

Preparation of the electrodes. The electrodes are so shaped that they can be attached to the perforated supporting plate. These electrodes are prepared from sections of steel wire (the E string of a violin) 5 cm long.

The sections of wire are immersed at one end in a weak solution of sulfuric acid, as a result of which this end is thinned to a diameter of less than 0.05 mm (Fig. 1, a). Stocks of electrodes are then dipped in tin of a high degree of purity. The other end of the steel wire is doubled over (see Fig. 1, b), twisted for a distance of 2 cm (see Fig. 1, c) and bent in a zigzag shape in order to give the wire a form of flat surface (see Fig. 1, d). At the beginning and end of the manipulated part of the electrode it is bent into a right angle (see Fig. 1, e).

In order to insulate the electrodes they are immersed once in a solution of organic glass of a syrupy consistency and dried at room temperature. The insulated electrodes are joined in pairs (see Fig. 1, f) with silk thread in their upper part and again immersed in the organic glass solution. The reliability of the insulation is tested with an ohm-meter. Next the points of the electrodes are carefully cleaned free from insulation by means of filter paper soaked in chloroform. After drying, the electrodes are tested for conductivity.

Operation of implantation of the perforated plate and electrodes. Under ether anesthesia a midline incision is made in the skin over the vault of the rat's skull. The skin is easily separated by blunt dissection from the periosteum and is retracted to the side with hooks; in this way the temporal muscles are exposed on the right and the left, enclosinga trapezoidal area of the vault of the skull which is covered only by periosteum. A piece of the supporting plate is cut out to the shape of this area. The perforated plate is fixed by 4 sutures to the temporal muscles. In young animals it is possible to insert the sutures directly into the skull behind the bony crest formed on each side by the area of the vault of the skull and the temporal bone. In this case the needle enters the bone at the level of the zygomatic process and emerges at the site of attachment of the temporal muscle

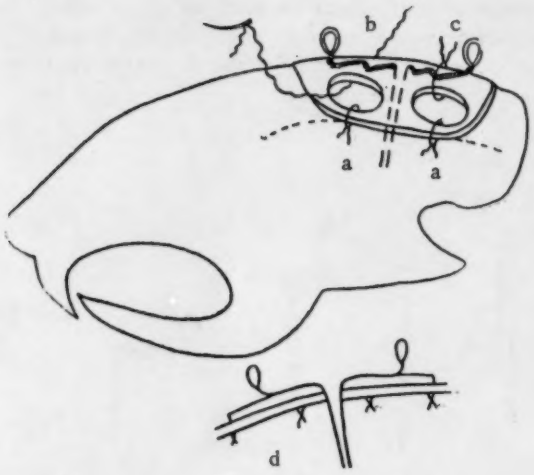

Fig. 2. Diagram showing the attachment of the perforated supporting plate and electrodes to the skull of a white rat. a) suture attaching the plate to the temporal muscle or the bony crest; b) ligature passing through a hole in the plate (between the plate and the skull); c) ligature fastened over the electrodes; d) diagram showing the position of the supporting plate and electrodes in sagittal section.

to the bones of the vault of the skull, i.e. to the bony crest (Fig. 2, a). At the time of operation the plate is readily bent to the shape of the skull. After the short time necessary for loss of the chloroform the plate hardens and henceforward keeps its acquired shape. The holes in the organic glass plate enable the attachment of a skin flap over the plate and restrict its horizontal displacement, since connective tissue grows through them.

By means of a No. 3 spherical dental drill a burr hole is made through the plate and at the same time through the underlying bone of the vault of the skull. Then, using a slightly curved needle 2 ligatures are passed under the plate (through its holes (see Fig. 2, b). The plate is coated in the midline with organic glass solution; the manipulated parts of the electrodes are also coated with the same solution. The ends of the

electrodes are then passed through the burr hole and carefully buried in the brain substance until the horizontal arms of the manipulated parts of the electrodes no longer remain poised over the surface of the supporting plate.* The electrodes are fixed to the plate by ligatures passed beneath it (see Fig. 2, c) and left for a few minutes in order to become adherent to the plate. After this time the periosteum is scarified by means of a sharp hook or a dental drill through the hole in the plate; this encourages further dense adhesion formation between the skin and the periosteum. The operation is concluded by suturing the skin; the emerging loops of the electrodes pass between the sutures.

The electrodes are thus firmly fixed to the animal's skull by their horizontal manipulated parts although these parts are not directly attached to the bones of the skull since they are joined only with the substance of the supporting plate which in the course of time becomes more and more firmly embedded.

To connect the electrodes to the stimulating or recording apparatus a fine lead is used, which is suspended on fine elastic, and a terminal hook by means of which the loop of the electrode is seized for convenient soldering of the lead thereto. In place of tinned steel electrodes, those of silver or constantan may be used; in this case the electrodes are soldered to the manipulated part of the steel wire.

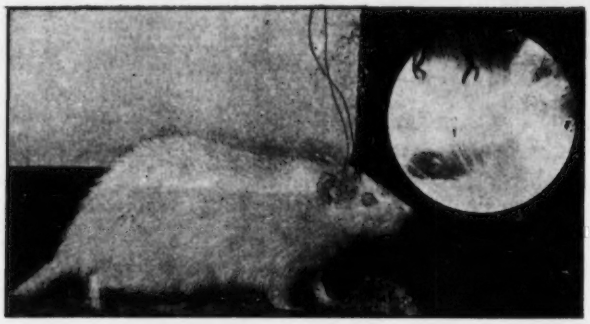

Fig. 3. A rat with electrodes in situ, 8 months after operation. On the magnified film of the head of the rat the electrode loops are seen among the growing fur.

We used the method described on white rats weighing from 50 to 200 g. The reliability of the attachment may be judged by the fact that during observations on these animals for 8 months the electrodes remained firmly in position (Fig. 3). Implantation of the electrodes in the brain of white rats by this method may also be used for stimulation of suitable nerve structures and for tapping potentials during experiments of long duration.

SUMMARY

A method of implantation of electrodes into the brain of white rats in a chronic experiment is described. Rats weighing from 50 to 200 g may be subjected to this operation. Stable fixation was followed up for 8 months.

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*Corresponding to the required depth of insertion of the electrodes, they are used in suitable lengths.
**In Russian.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Ekspti. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Ekspti. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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